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(54) Title: POLYVALENT PRESENTER COMBINATORIAL LIBRARIES AND THEIR USES

(57) Abstract

The methods of the present invention provide for the synthesis and screening of combinatorial libraries of polyvalent presenters. The polyvalent presenters formed using the methods of the present invention generally have the formula (I);: $R^1\{-R^3\}_m$, wherein R^1 is a framework component, R^3 is a functional group component, and m is an integer having a value greater than ten and which is selected such that the presented functional groups can interact with a collection of greater than ten target binding sites. The framework component must b3e at least 10 KDa MW of sufficient means hydrodynamic radius to span the distance between adjacent receptors of the target, (ie.e, about 100Å or greater). These dimensions permit the plurality of functional groups attached to the framework to simultaneously bind to the target receptors (e.g., cell surface receptors). In some embodiments, the polyvalent presenters have the formula (II): $R^1\{-R^2(-R^3)_m\}_n$, wherein R^1 and R^3 area s defined above, m is an integer having a value greater than ten and which is selected such that the presented functional groups can interact with a collection of greater than ten target binding sites. In other embodiments, ancillary groups are prsent in the polyvalent presenters of the present invention, the ancillary group imparting or latering a characteristic(s) of the polyvalent presenter. Properties which can be imparted and–or modified include, for example, solubility (in water, fats, lipids, biological fluids, etc.), hydrophobicity, hydrophilicity, charge framework flexibility, antigenicity, molecular size, molecular weight, biocompatibility, immunogenicity, stability, *in vivo* half-life, *in vivo* distribution, strength of binding to the polyvalent target, etc.

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POLYVALENT PRESENTER COMBINATORIAL
LIBRARIES AND THEIR USES

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RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No.
60/043,288, filed April 11, 1997 and U.S. Provisional Application Serial No. 60/043,918,
filed April 15, 1997, which is incorporated herein by reference. This application is also
20   related to U.S. Provisional Application Serial No. 60/043,781, filed April 11, 1997 and to
U.S. Provisional Application Serial No. 60/043,826, filed April 14, 1997. The contents of the
aforementioned applications are incorporated herein by reference.

1.   BACKGROUND OF THE INVENTION

25   Polyvalent interactions are ubiquitous in biological systems. They are
characterized by the simultaneous binding of multiple ligands on one biological entity (e.g., a
molecule, a surface, etc.) to multiple receptors on another. Ligands include molecules that
convey information in biological systems or that are acted on by proteins. Examples of types
of ligands are drugs, hormones, signaling molecules, toxins, enzyme substrates, bioregulators,
30   neurotransmitters and lymphokines. Receptors include molecules that receive the information
from the ligands. Most receptors are proteins and include, for example, protein receptors,
antibodies and enzymes. Some receptors are nucleic acids and include the regulatory regions
of DNA and RNA. Membranes represent yet another class of receptors.

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Many drugs are ligands that interact with a single receptor (some drugs are receptors that interact with a single ligand).

Polyvalent interactions have a number of characteristics that monovalent interactions do not. In particular, polyvalent interactions can be collectively much stronger than the corresponding monovalent interactions, and they can exert agonistic or antagonistic effects on biological processes by mechanisms that are fundamentally different from those available in monovalent systems.

Polyvalency typically is involved in interactions that occur at cell surfaces and/or interactions that involve groups of receptors or clusters of receptors. Polyvalency can also be important in macromolecular interactions involving multiple points of attachment simultaneously. The idea that many biological systems interact through multiple simultaneous molecular contacts is familiar; it has, however, become a new focus of inquiry in molecular biochemistry as the importance of interactions involving multiple proteins and ligands has begun to be unraveled. The possibility that multiple simultaneous interactions have *unique* collective properties that are qualitatively different from properties displayed by their constituents, interacting monovalently, suggests new strategies for the design of drugs and for research reagents for biochemistry and biology. Enhancing or blocking collective or polyvalent interactions may benefit from (or require) strategies fundamentally different from those used in monovalent molecular interactions.

The concept of polyvalency has been explored. For instance, studies have been conducted elucidating the mechanism for adhesion of the influenza virus to the surface of cells (*see*, Whitesides, *et al.*, *J. Med. Chem.* 1995, 38, 4179-4190). In addition, Kiessling, *et al.* (*Chemistry and Biology*, 1996, Vol. 3, No. 2, 71-77), created structural templates for the generation of multivalent carbohydrate displays to study and modulate biological recognition events. Kiessling, *et al.* viewed these as "chemical tools available to explore multivalent protein-saccharide interactions." In a section of the reference entitled "Where do we go from here?," Kiessling, *et al.* set forth that "[a]s well as being useful to explore biological recognition processes, multivalent carbohydrates can be used as probes for biological function". Matrosovich (*FEBS LETTERS*, 1989, *252(1)*:1-4) proposed the use of polyvalent inhibitors for inhibiting microbial attachment. Matrosovich sets forth that "[t]o

produce such polyvalent structures, one might utilize some principles of the design of the well-known 'drug delivery systems', for example, coupling the monovalent inhibitory active molecules in multiple copies to soluble biocompatible polymers or microparticulate carriers". Matrosovich further sets forth that" [t]he correctness of and the prospects for the practical use of this approach to the design of antimicrobial agents could be evaluated in the future...".

SUMMARY OF THE INVENTION

To date, "high affinity, specific binding events have dominated most thinking about receptor-ligand interactions" (see page 71 of Kiessling *cited supra*). Typically, attempts to optimize receptor-ligand interactions have focused on the binding capability or the specificity of a particular ligand with a particular receptor- binding site in an individual binding event. For example, a ligand was selected for interaction based upon its known favorable binding capability or a ligand believed to be a weak binder was avoided or chemically modified to enhance its binding capability before being selected for use.

The present invention is based, at least in part, on our viewing of receptor-ligand interactions and polyvalency in a non-conventional, global manner based on an understanding of how a multicomponent polyvalent presenter interacts with a collection of target binding sites. This differs from the conventional manner of viewing such interactions on a more individualistic basis as a plurality of separate uncoupled receptor-ligand interactions. The non-conventional, global manner of viewing receptor-ligand interactions and polyvalency in biological systems has led us to the realization that polyvalency can be used as a basis, *e.g.*, a primary basis, for rational drug design and further, that polyvalent agents have universal applications for treating many different diseases or conditions. The non-conventional, global manner of viewing receptor-ligand interactions has led us even further to the realization that the choice of a particular ligand based on its individual binding capability need not be the most important parameter in designing a polyvalent drug. For instance, weakly binding ligands can be used in polyvalent presenters of the present invention. Based, in part, on the foregoing, the present invention provides methods for preparing and screening combinatorial libraries, *i.e.*, arrays, of polyvalent presenters. The

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polyvalent presenters are generally formed by constructing and arranging a plurality of functional group components R^3 (*e.g.*, ligands), on a plurality of framework components (*e.g.*, polymeric backbones), thereby forming an array of polyvalent presenters. Once formed, the array of polyvalent presenters can be screened for useful properties, and the polyvalent

5   presenters identified as having useful properties can, in turn, be used to treat a disease or condition.

Generally, the methods of the present invention provide for the synthesis and screening of combinatorial libraries of polyvalent presenters. The polyvalent presenters formed using the methods of the present invention generally have the formula :

10                                     $$R^1\{-R^3\}_m \qquad \qquad (I)$$

wherein: R^1 is a framework component , R^3 is a functional group component, and m is an integer having a value greater than ten and which is selected such that the presented functional groups can interact with a collection of greater than ten target binding sites. The framework component must be at least 10 KDa MW of sufficient mean hydrodynamic radius to span the

15   distance between adjacent receptors of the target, (i.e. about 100Å or greater). These dimensions permit the plurality of functional groups attached to the framework to simultaneously bind to the target receptors (e.g., cell surface receptors). In some embodiments, the polyvalent presenters have the formula:

$$R^1\{-R^2(-R^3)_m\}_n \qquad \qquad (II)$$

20   wherein: R^1 and R^3 are as defined above, m is an integer having a value equal to one or greater, R^2 is a spacer or linker group and "n" is an integer having a value greater than ten and which is selected such that the presented functional groups can interact with a collection of greater than ten target binding sites. In other embodiments, ancillary groups are present in the polyvalent presenters of the present invention, the ancillary group imparting or altering a

25   characteristic(s) of the polyvalent presenter. Properties which can be imparted and/or modified include, for example, solubility (in water, fats, lipids, biological fluids, *etc.*), hydrophobicity, hydrophilicity, charge, framework flexibility, antigenicity, molecular size, molecular weight, biocompatability, immunogenicity, stability, *in vivo* half-life, *in vivo* distribution, strength of binding to the polyvalent target, *etc.*

As noted, the methods of the present invention involve the formation of combinatorial libraries that consist of an array of synthetic polyvalent presenters, wherein the polyvalent presenters in the array differ from one another in terms of their compositions, structures, properties, functions, *etc.* In making the arrays of polyvalent presenters, one can

5   vary, *inter alia*, the chemical structure of the framework component, the chemical structure of the functional group components, the chemical structure of the ancillary groups, the chemical structure of the spacer groups, the chemical nature of the framework components, the chemical nature of the functional group components, the chemical nature of the ancillary groups, the chemical nature of the spacer groups, the amount of framework component

10   delivered, the amount of functional group component delivered, the amount of ancillary group component delivered, the amount of spacer group delivered, the number and/or amount of different framework components delivered, the number and/or amount of different functional group components delivered, the number and/or amount of different ancillary groups delivered, the number and/or amount of different spacer groups delivered, the nature

15   and number of the linkages between the various components (*e.g.*, the nature of the linkages between the spacer and framework components), the relative position(s) of attachment of functional groups to the framework and/or spacer components, the reaction parameters (*e.g.*, reactant solvents, reaction temperatures, reaction times, reaction initiators, reaction catalysts, the atmosphere in which the reactions are carried out, the pressure at which the reactions are

20   carried out, the rates at which the reactions are quenched, *etc.*); the stoichiometry of the various components; the order in which the different components are delivered, *etc.*

Accordingly, in one embodiment, the invention provides a method of making an array of polyvalent presenters, the method comprising: (a) delivering a first activated framework component of a first polyvalent presenter and a first activated framework

25   component of a second polyvalent presenter to first and second reaction vessels; and (b) delivering a first functional group component of the first polyvalent presenter and a first functional group component of the second polyvalent presenter to the first and second reaction vessels for reaction therein, thereby forming the array of at least two different polyvalent presenters. This process is optionally repeated, with additional components (*e.g.*,

30   framework components, functional group components, ancillary groups, spacer groups, *etc.*)

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and/or different reaction parameters (*e.g.*, different reaction temperatures, reaction catalysts, reaction solvents, *etc.*), to form a diverse array of polyvalent presenters.

In another embodiment, the present invention provides a method of making an array of polyvalent presenters, the method comprising: (a) delivering a first monomer of a
5   first polyvalent presenter and a first monomer of a second polyvalent presenter to first and second reaction vessels; (b) delivering a first functional monomer of the first polyvalent presenter and a first functional monomer of the second polyvalent presenter to the first and second reaction vessels; and (c) copolymerizing the first monomer and the first functional monomer of the first polyvalent presenter and the first monomer and the first functional
10   monomer of the second polyvalent presenter; thereby forming the array of at least two different polyvalent presenters. This process is optionally repeated, with additional components (*e.g.*, monomers, functional monomers, ancillary groups, spacer groups, *etc.*) and/or different reaction parameters (*e.g.*, different reaction temperatures, reaction catalysts, reaction solvents, *etc.*), to form a diverse array of polyvalent presenters.

15   In yet another embodiment, the present invention provides a method of making an array of polyvalent presenters, the method comprising: (a) delivering a first monomer of a first polyvalent presenter and a first monomer of a second polyvalent presenter to first and second reaction vessels; (b) delivering a first functional monomer of the first polyvalent presenter and a first functional monomer of the second polyvalent presenter to the
20   first and second reaction vessels; and (c) copolymerizing the first monomer and the first functional monomer of the first polyvalent presenter and the first monomer and the first functional monomer of the second polyvalent presenter; and (d) delivering a second monomer or a second functional monomer of the first polyvalent presenter and a second monomer or a second functional monomer of the second polyvalent presenter to the first and
25   second reaction vessels; thereby forming the array of at least two different polyvalent presenters. This process is optionally repeated, with additional components (*e.g.*, monomers, functional monomers, ancillary groups, spacer groups, *etc.*) and/or different reaction parameters (*e.g.*, different reaction temperatures, reaction catalysts, reaction solvents, *etc.*), to form a diverse array of polyvalent presenters.

In another aspect, the present invention provides an array of polyvalent presenters. It is preferred that the polyvalent presenters of the array are confined to predetermined spatially separate regions on a substrate or in separate reaction vessels. Such an array can consist of between 2 and 10^6 different polyvalent presenters.

5   In one embodiment of the present invention, the construction and arrangement of groups R^3 on R^1 is based on one or more desired biological properties and/or therapeutic benefits.

Once formed, the array of polyvalent presenters can be screened, sequentially or in parallel, for biological and pharmacological activities related to therapeutic uses.

10   Screening can be performed *in situ* or, alternatively, the polyvalent presenters can be screened in other than an *in situ* manner (*e.g.*, the polyvalent presenters can be removed from the substrate and then screened). Properties that can be screened for include, but are not limited to, the following: biological activities, binding affinities, pharmacological properties, oral bioavailabilities, circulatory half-lives, agonist activities, antagonist activities, solubilities, *etc.*

15   Once identified, the polyvalent presenters having useful properties can be prepared on a large-scale, although not necessarily by the above-described methods.

The polyvalent presenters having useful properties can be used to treat a disease or condition.

Other features, objects and advantages of the invention and its preferred

20   embodiments will become apparent from the detailed description which follows.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic depicting both a monovalent and polyvalent reaction.

FIG. 2 is a schematic depicting the "blanketing" of a polymeric polyvalent

25   presenter(s) over a collection of binding sites and an array of target binding sites B.

FIG. 3: a synthesis of polymeric polyvalent galactosides. (a) $CH_2 = CHCH_2OH$, $BF_3.Et_2O$, CH_2Cl_2, 0°C to ~ 20°C, 34 h, 91%; (b) LiOH, MeOH:H_2O (2:1), ~20°C, 12h, ~100%; (c) hv (254 nm), $HSCH_2CH_2NH_2$, 4,4'-azobis(4-cyanovaleric acid), H_2O-MeOH (10:1), 10 h, 67-74%; (d) $CH_2 = CHCH_2SiMe_3$, $BF_3.Et_2O$, MeCN, 0°C, 40 h, 71%;

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(e) RNH_2, i-Pr_2NEt, N,N-dimethylformamide, ~20°C, 2 days; then 1.0 M NaOH; dialysis: RNH_2 = Gal-β_O-L_1NH_2, Gal-α_C-L_2NH_2.

FIG. 4: plots of agglutination (RCA-induced) inhibition (K_i^{HA}) of polymeric polyvalent galactoside versus mole fraction of Gal (χ^{Gal}) of the polymer: against RCA_{120} (a); RCA_{60} (b). As controls, other polymers presenting non-galactoside chains are included: pAA(GlcNAc-β), pAA presenting GlcNAc-β_O-L_1NH_2 as amide side chains; pAA(NeuAc-α), pAA presenting N-acetylneuraminic acid (NeuAc)-α_C-L_2NH_2 as amide side chains; pBMA(NeuAc-α), pBMA presenting NeuAc-α_C-L_2NH_2 as amide side chains. Arrows shown on the two plots signify that the HAI activities of some of the materials that were tested could not be observed at the indicated concentrations, and the values of K_i^{HA} of the compounds are expected to be higher than those indicated.

FIG 5: (a) Generation of pMVMA(NeuAc) using quasi-solid phase reaction; (b) generation of pMVMA(NeuAc;R) using quasi-solid phase reaction; (c) generation of pAA(Gal) using quasi-solid phase reaction; (d) generation of pBMA(Gal) using quasi-solid phase reaction.

FIG. 6: (a) generation of pAA(SLe^x) using quasi-solid phase reaction; (b) generation of pAA(Bacitracin;R) using quasi-solid phase reaction.

II. *DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS*

I. *COMBINATORIAL METHODS*

Combinatorial chemistry is becoming an important tool both in the search for new pharmacological leads and for lead compound optimization (Gallop *et al.*, *J. Med. Chem.* 1994, 37, 1233-1251; Gordon, E.M. *et al.*, *J. Med. Chem.* 1994, 37, 1385-1401; Thompson and Ellman, *Chem. Rev.* 1996, 96, 555-600). The present invention provides methods for preparing and screening combinatorial libraries of polyvalent presenters. The polyvalent presenters are generally formed by constructing and arranging a plurality of functional group components, *i.e.*, groups R^3 (*e.g.*, ligands), on a plurality of framework components (*e.g.*, a polymeric backbone), thereby forming an array of polyvalent presenters. Once formed, the array of polyvalent presenters can be screened for useful properties, and the

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polyvalent presenters identified as having useful properties can, in turn, be used to treat a disease or condition.

The methods of the present invention provide for the synthesis and screening of combinatorial libraries of polyvalent presenters. The polyvalent presenters formed using the methods of the present invention generally have the formula:

$$R^1\{-R^3\}_m \qquad\qquad (I)$$

wherein:

R^1 is a framework component, R^3 is a functional group component, and m is an integer having a value greater than ten and which is selected such that the presented functional groups can interact with a collection of greater than ten target binding sites. The framework component must be of sufficient mean hydrodynamic radius to span the distance between adjacent receptors of the target, and will be about 100Å or greater. These dimensions permit the plurality of functional groups attached to the framework to simultaneously bind to the target receptors (e.g., cell surface receptors). In some embodiments, the polyvalent presenters have the formula:

$$R^1\{-R^2(-R^3)_m\}_n \qquad\qquad (II)$$

wherein: R^1 and R^3 are as defined above, m is an integer having a value equal to one or greater, R^2 is a spacer or linker group and "n" is an integer having a value greater than ten and which is selected such that the presented functional groups can interact with a collection of greater than ten target binding sites. As described herein, numerous spacer groups with various linkages can be present in the polyvalent presenters of the present invention. As well, ancillary groups, which are described in detail herein, are present in the polyvalent presenters of the present invention for imparting or altering a characteristic(s) of the polyvalent presenter.

The term "monomer," as used herein, defines a small molecule (e.g., MW < 1000 daltons), oligomer or polymer which incorporates within its structure one or more polymerizable moieties. Suitable polymerizable moieties include, but are not limited to, carbon-carbon and carbon-heteroatom multiple bonds (e.g., vinyl, acrylic, urethane, cyano), ethylene oxide, natural- and pseudo-amino acids, orthoesters, anhydrides, aldehydes and

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oligomers and polymers bearing these or other polymerizable moieties. Additional polymerizable moieties are known, and readily available to those of skill in the art.

The term "functional monomer," as used herein, defines a molecule which incorporates within its structure both a polymerizable moiety and a functional group as

5   defined herein. The polymerizable moieties can be small molecules (e.g., MW < 1000 daltons) or can themselves be polymers or oligomers.

As described herein, the methods of the present invention involve the formation of combinatorial libraries which consist of an array of synthetic polyvalent presenters, wherein the polyvalent presenters in the array differ from one another in terms of

10   their compositions, structures, properties, functions, etc. In making the arrays of polyvalent presenters, one can vary, inter alia, the chemical structure of the framework component, the chemical structure of the functional group component, the chemical structure of the ancillary group, the chemical structure of the spacer group; the chemical nature of the framework component, the chemical nature of the functional group component, the chemical nature of

15   the ancillary group, the chemical nature of the spacer group; the amount of framework component delivered, the amount of functional group component delivered, the amount of ancillary groups delivered, the amount of spacer group delivered; the number and amount of different framework components delivered, the number and/or amount of different functional group components delivered, the number and/or amount of different ancillary groups

20   delivered, the number and/or amount of different spacer groups delivered; the nature and number of the linkages between the various components (e.g., the nature of the linkages of the spacer group); the reaction parameters (e.g., reactant solvents, reaction temperatures, reaction times, reaction initiators, reaction catalysts, the atmospheres in which the reactions are carried out, the rates at which the reactions are quenched, etc.); the stoichiometry of the

25   various components; the order in which the different components are delivered, etc. The various reactant components (e.g., the framework component, the functional group component, the ancillary group, the spacer group, etc.) and the various methodologies which can be employed are described in greater detail hereinbelow.

Generally, the array of polyvalent presenters is prepared by delivering the

30   various components to the reaction regions. As explained hereinbelow, the components can

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be sequentially or simultaneously delivered to the reaction vessels, *i.e.*, predefined regions on
the substrate, using any number of a number of different delivery techniques. For instance,
the various components can be delivered into the reaction regions of interest from a dispenser
in the form of droplets or powders. Suitable dispensers include, but are not limited to,

5   micropipettes, mechanisms adapted from ink-jet printing technology and electrophoretic
pumps.

        A.   The Framework Component

                The group R^1 of Formulae I and II, *supra*, represents a framework component

10   which is preferably, non-toxic, minimally antigenic and sufficiently functionalized (or
capable of functionalization) to permit attachment to a multitude of functional groups,
ancillary groups and/or spacer groups through various linkages (including, *e.g.*, cleavable,
hydrolyzable, biocompatible and biodegradable linkages) where appropriate.
The term "framework component" generally refers to a support structure or backbone of

15   sufficient mean hydrodynamic radius to span the distance between adjacent receptors on the
target, and will be about 100Å or greater. These dimensions permit a plurality of functional
groups (i.e., greater than 10) attached to the framework to simultaneously bind to the target
receptors (e.g., cell surface receptors). One skilled in the art will know that the mean
hydrodynamic radius of a polymer can be estimated crudely using standard statistical

20   mechanical methods. The attachment can be by any means that allows the polyvalent display
of the functional groups on the framework components. In some embodiments, the ·
"backbone" of the framework component can further comprise spacers for joining monomer
units of the framework component together. Specific backbone spacers are discussed in
detail below in the context of polymeric frameworks. In certain embodiments, the spacers ·

25   are cleavable, *e.g.* the spacers can be hydrolytically, chemically, photochemically or
enzymatically labile.

                The types of framework components useful within the polyvalent presenter
are those capable of having functional groups attached thereto and capable of polyvalently
presenting the functional groups. Examples of framework components suitable for use in the

30   methods of the present invention include, but are not limited to, polymers, liposomes,

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micelles, colloids, dendrimers, biological particles and non-biological particles (*e.g.*, silica beads, polymeric beads, gels, *etc.*). These various types of framework components are discussed briefly below and in more detail even further below under the description of covalent and noncovalent frameworks. The detailed description of each of these framework

5   components are provided under the headings of covalent and noncovalent framework components only for ease of discussion and should not be construed as limiting the scope of frameworks. It should be understood that the present invention is intended to encompass all types of frameworks capable of polyvalently presenting functional groups, *i.e.*, groups R^3, as described herein.

10   The terms "polymer" and "polymeric" are art-recognized terms and include reference to a structural framework comprised of repeating monomer units, which is in the context of the present invention, polymers are presenters if they are comprised of at least 100 subunits and are capable of polyvalently presenting R^3 groups such that the treatment of a disease or condition occurs. The terms also include reference to homopolymers and

15   copolymers. Linear polymers, branched polymers and cross-linked polymers are also included by the terms "polymer" and "polymeric."

The terms "liposome," "micelles," and "colloids" are art-recognized terms. These terms also include reference to the derivatized versions, *e.g.*, liposome derivatives, cross-linked liposomes, *etc.*

20   The term "dendrimer" is also an art-recognized term and includes reference to a specific subclass of branched polymers that possess multiple generations. In dendrimers, each generation creates multiple branch points.

The term "biological particle" includes reference to both covalent molecules, *e.g.*, sugars, proteins, lipids, small molecules, protein aggregates and nucleic acids, and

25   noncovalent particles, *e.g.*, modified cells (*e.g.*, which have been derivatized, modified chemically or transfected with an exogenous nucleic acid), or modified viruses, *e.g.*, viral particles. The use of "biological particles" as framework components is distinguished from such particles as they occur in their natural state because the subject framework components are modified to polyvalently present functional groups R^3.

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The term "oligopeptide" and "polypeptide" are a class of compounds composed of amino acid units chemically bound together with amide linkages. A polypeptide is thus a polymer of amino acids, forming chains that may consist of as many as several thousand amino acid residues.

5   *1. Covalent Framework Components*

In one embodiment, the monomeric units of a framework component can be joined covalently. Exemplary covalent frameworks include, but are not limited to, cross-linked liposomes, biological particles (*e.g.*, sugars, proteins, peptides, lipids, or small molecules) and polymeric materials (*see, e.g.*, Siraganian, *et al.*, *Immunochem.* 1975, *12*,

10   149-155; Wofsy, *et al.*, *J. Immunol.*, 1978, *121*, 593-601; Barlocco, *et al.*, *Farmaco*, 1993, *48*, 387-96; Castagnino, *et al.*, *Jpn. Heart J.*, 1990, *31*, 845-55; Costa, *et al.*, *Biochem. Pharmacol.*, 1985, *34*, 25-30; Dembo, *et al.*, *J. Immunol.*, 1979, *122*, 518-28; Holliger, *et al.*, *Proc. Natl. Acad. Sci. U.S.A*, 1993, *90*, 6444-8; Piergentili, *et al.*, *Farmaco*, 1994, *49*, 83-7; Portoghese, *et al.*, *J. Med Chem.*, 1991, *34*, 1292-6; Kizuka, *et al.*, *J. .Am. Chem. Soc.*, 1987,

15   *30*, 722-6).

In certain embodiments, proteins, *e.g.*, albumin, can be used as a framework component for presenting large numbers of groups (Roy, *et al.*, *Can. J. Chem.*, 1990, *68*, 2045-2054), thereby mimicking natural glycoproteins.

In other embodiments, polymers can be used as the framework component for

20   the polyvalent presenters. Polymers are a versatile framework system (*see, e.g.*, Spaltenstein, *et al.*, 1991, *J. Am. Chem. Soc.*, 113:686; Mammen, *et al.*, 1995. *J. Med. Chem.*, 38:4179). In a preferred embodiment, the groups R^3 of the present invention are attached through a spacer group (or, interchangeably, a linker group) to a framework component comprising a polymeric backbone. In certain embodiments, reactive or activated polymers can be used in

25   the "framework component" of the present invention as described in more detail below.

Polymers can be purchased from commercially available sources or, alternatively, they can be prepared using methods known to those of skill in the art (*See, e.g.*, Sandler, S. R.; Karo, W., *Polymer Syntheses*, Harcourt Brace: Boston, 1994; Shalaby, *et al.*, *Polymers of Biological and Biomedical Significance (ACS Symposium Series 540*, American

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Chemical Society: Washington, DC, 1994). Moreover, those of skill in the art will appreciate that polymeric, polyvalent presenters are easily, rapidly and convergently synthesized (see, e.g., Spaltenstein, et al., 1991, J. Am. Chem. Soc. 113:686; Mammen, et al., 1995, J. Med. Chem. 38:4179).

5   In addition, polymers provide a number of advantages as the framework component because the characteristics of the polymer can be varied, modulated and controlled as desired. For instance, characteristics which can be varied and controlled include, but are not limited to, size, gross geometry, dispersity, charge, conformal flexibility; solubility; hydrophilicity; modulation of conformation and flexibility in solution through

10   variations in temperature and ionic strength, etc. As such, the use of polymers readily allows for the modulation of various physical properties of the presenter. For instance, the polymeric systems can be designed or selected to allow multiple groups R^3 to bind simultaneously to multiple target binding sites with minimal unfavorable strain. Additionally, the characteristics of the polymers can be designed to vary the flexibility of the

15   polymer, the distance between the functional groups (e.g., bioactive sidechains), the length of the spacer group or linker between the polymer backbone and the functional groups, etc.

The chemistry of high molecular weight polymers is a well-developed science, and organic polymers provide a very important class of compounds to use for polyvalent presentation. Such compounds have high molecular weights, can present very

20   large numbers of copies of the functional group and can present more than one functional group simultaneously. Moreover, their transport across biological membranes is typically limited and, thus, their lifetime in particular compartments can be controlled in vivo. As noted above, polymeric frameworks offer a variety of easily synthesized macromolecules, and access to a wide range of biological properties and activities.

25   In preferred embodiments, modified polymeric materials for use in the present invention have low antigenicity and low toxicity. In preferred embodiments, polymeric frameworks can be selected to be compatible with water, to be capable of having varied molecular weight and to be capable of having a range of different groups (e.g., functional groups, ancillary groups, spacer groups, etc.) attached to the polymer backbone. Polymer

30   backbones of the present invention can also be selected for ease of synthesis.

Intrinsically biocompatible polymers containing functional groups appropriate for the addition of sidechains are preferred (Shalaby, *et al., Polymers of Biological and Biomedical Significance* (ACS Symposium Series 540), American Chemical Society: Washington, DC, 1994). Exemplary polymers include, but are not limited to, polyethylene oxide or polyethyleneglycol (Harris, J. M., *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*, Plenum: New York, 1992; Horton, D., *Advances in Carbohydrate Chemistry and Biochemistry*, Academic Press: San Diego, 1995) as well as polyamides, polyesters, proteins, derivatives of acrylamide and N-vinylpyrollidone, linked oligomers of oligoethylene glycol, linked oligomers of dextran and others.

Other preferred framework components for use in the methods of the present invention have demonstrated utility, for example, as plasma extenders, drug excipient or binders, food additives or as inert or erodible materials used *in vivo*. For example, poly(ethylene glycol), poly(lactic acid), poly(glycolic acid) and poly(vinyl pyrrolidone) can be used as the framework component in the methods of the present invention.

Preferred polymers contain reactive or activatable groups, such as carboxylic acids and anhydrides. A number of synthetic and naturally-occurring polymers containing carboxylic acid functionality (or capable of being suitably modified) have been used *in vivo*. Such polymers are capable of forming covalent linkage with a presented R^3, *e.g.*, an activated ester bond which when reacted with an amine can facilitate linkage of groups as described herein. Polymers containing internally cyclized carboxylic acid functionality, such as anhydride or succinimide groups, are especially desirable. Other preferred polymers include, but are not limited to, subunits derived from maleic anhydride and malic acid. Exemplary copolymers include, but are not limited to, styrene-maleic anhydride and alpha-olefin-maleic acid copolymers (such as divinylether-maleic acid). In other embodiments, sodium carboxymethylcellulose, chondroitin sulfate and poly(methacrylate/acrylate) materials can be used. In still other embodiments, polymers without activated carboxylic acids can be used, such as dextran sulfate. Other exemplary polymeric framework components are shown in Table 1.

It will be appreciated by the skilled artisan that any polymeric material that is capable of presenting a plurality of functional groups can be suitable for use in the present

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invention. Polymers can be modified, *e.g.*, as described above or by derivatization, *e.g.*, with bifunctional cross-linking reagents, to provide functionalities suitable for attaching and presenting functional groups, as described in more detail below.

Table 1. Exemplary polymers

Poly(ethylene glycols)	Poly(ethylene-vinyl acetate)
	Poly(acrylamides)
Poly(amides)	Poly(urethanes)
poly(peptides)	Poly(methacrylates)
	Poly(acrylates)
Poly(amino acids)	Poly(maleic acid copolymers)
poly(aspartic acid)	Poly(anhydrides)
	Poly(orthoester)
Poly(glutamic acid)	
poly(lysine), others	_____
proteins(gelatins)	
Poly(esters)	
poly(lactic acid), polylactide	
poly(glycolide)	
poly(caprolactone)	Copolymers
poly(tartrate)	with degradable linkage
	with groups for attachment
Polysaccharides	
cellulose	Encapsulation
alginates	for protection
starch	for targeting
Dextran derivatives	
Poly(N-vinylpyrrolidone)	

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More narrowly defined classes include linear polypeptides and oligopeptides containing both essential and nonessential amino acids, including, for example, lysine, ornithine and glutamic acid, and any other polypeptides and oligopeptides which have one or more terminal amino groups and are available in the desired molecular weights. A further

5   class is that of branched synthetic oligopeptides and polypeptides, such as branched dendrimers of amino acids such as lysine, the dendrimers being readily synthesized in a controlled manner using conventional techniques to yield a controlled number of functional groups. Polysaccharides, such as dextrans, starches and celluloses, are a still further class, and simple non-biological polymers, such as polyethyleneimine and polyacrylic acid, are yet

10   a further class. Derivatized analogs of the polymers of these classes include polymers modified to contain selected functionalities so as to permit attachment of the functional group component, ancillary group and/or spacer group as described herein. Examples include, but are not limited to, polymers such as poly(aminopropyl)dextran and hydroxyethyl starch, and copolymers such as poly(styrene-co-maleic anhydride).

15   *Backbone spacers*. In certain embodiments it will be desirable to include connecting moieties between the monomeric subunits of the backbone. Exemplary "backbone spacers" can include hydrocarbon, carbamate, amide, ether, thioester, thioether, carbonate and ester connections. In certain embodiments, the backbone moieties can be linked with a cleavable linker. The lifetime of the polyvalent material *in vivo* may depend in

20   part on its molecular dimensions. By placing linker groups between medium-sized oligomers, and by controlling the *in vivo* stability of these linking groups, the lifetime of the polymers *in vivo* can be controlled. The use of non-functionalized, degradable connectors in the polymer backbone can therefore be used to assist clearance of the polymer. The cleavable spacer will generally be different from that which is used to link the polymer

25   backbone to the functional group, R^3. Such a cleavable linkage will cause the formation of smaller, polymeric functionalized fragments that will be small enough to clear through the kidney. Degradable linkers can include, e.g., hydrolytically labile linkers including ester, carbonate or oxalate groups.

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2. Noncovalent Framework Components

A plurality of functional group components, ancillary groups and/or spacer groups can also be joined to a non-covalent framework. Exemplary noncovalent frameworks include, but are not limited to, liposomes, micelles, colloids, protein aggregates, modified cells, and modified viral particles. For example, functional and/or ancillary groups can be tethered to the head groups of molecules in liposomes, membranes or surfaces (*see, e.g.,* Kingery-Wood, *et al., J. Am. Chem. Soc.,* 1992, *114,* 7303-7305; Spevak, *et al., J. Am. Chem. Soc.,* 1993, *115,* 1146-1147; Spevak, *et al., J. Med Chem.,* 1996, *39,* 1018-1020).

Liposomes and micelles are art-recognized terms and include macroscopic particles made up of aggregates of surfactants. In one embodiment, the polyvalent presenter can present groups on a liposome or micelle (Spevak, *et al., Am. Chem. Soc.,* 1993, *115,* 1146-1147; Charych, *et al., Chem. & Biol.,* 1996, *3,* 113-120). This system often mimics the shape of the target cell, and can be designed to present a surface that closely matches that of the target cell both in terms of group type and group density. For example, lipid molecules containing functional groups (*e.g.,* neuraminic acid (NeuAc)) as polar head groups can be reconstituted into liposomes. Liposomes have favorable bio-compatibility and are fairly easy to synthesize. In addition, liposomes can be designed to act as sensors. For instance, polymerized liposomes can be used to sense the conformational change of the liposome by displaying the change in UV/vis absorption of the internal chromophore (*e.g.,* a cross-linked polydiacetylene) of the membrane. For example, the binding of virus to the liposome can be detected by the shift in color (blue to red) as is art-recognized.

In other embodiments, biological particles including, for example, modified cells or modified viruses can be used as the framework component for polyvalent presentation of the functional group. Thus, proteins, peptides, polysaccharides, fragments of cell membranes, or modified intact cells (*e.g.,* erythrocytes), modified bacterial cells or modified viruses can be used as the framework component in certain embodiments.

3. Activated Framework Component

As used herein, the term "activated framework" refers to the framework component as described above, including both covalent and noncovalent framework

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components, that contain functionality that can be activated by means of an "activating group," and subsequently reacted with at least one functional group, ancillary group and/or spacer group. Appropriate functionality includes, for example, carboxyl (acid form and salts), hydroxyl, sulfhydryl, amide, carbamate, amino, ketone, aldehyde, olefin, aromatic, *etc.*

5   The polymers may be activated prior to exposing them to the functional group ("preactivation"), or may be activated in the presence of the functional group ("*in situ*").

The activation step can entail derivatizing the polymer with groups capable of undergoing reactions with nucleophiles or electrophiles. Further, it is within the scope of the present invention to activate polymers such that they are able to participate in dipolar

10   additions (*e.g.*, 1,3- and 1,4-dipolar addition), cycloaddition reactions (*e.g.*, Diels-Alder type reactions) and polymerization reactions by cationic, anionic or radical initiated mechanisms.

Carboxyl groups may be activated for reaction with nucleophiles by the use of, for example, cyclic or linear anhydrides, activated esters (*e.g.*, N-hydroxysuccinimide, nitrophenol, 4-hydroxy-3-nitrobenzene sulfonic acid, *etc.*), acid chlorides, imidazolides (*e.g.*,

15   from carbonyldiimidazole), carboxylic acid and esters. Carboxylic acid containing polymers may also be activated by forming adducts between the carboxyl group and agents such as, dicyclohexylcarbodiimide, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, alkyl chloroformates, chlorosilanes, pyridinium salts and Bu_3N, *etc.*

The selection of appropriate activating groups for the carboxyl functionality

20   will be apparent to those of skill in the art. It will be similarly apparent to those of skill in the art which reaction systems will be amenable to, or will require, *in situ* activation or preactivation.

Hydroxyl groups may be activated by the use of carbonates formed by reaction with, for example, alkyl or acyl haloformates (*e.g.*, *iso*-butylchloroformate, p-

25   nitrophenylchloroformate, *etc.*), cyanogen bromide or phosgene. In aspects utilizing polymers containing vic-diol groups (*e.g.*, dextran and other polysaccharides) oxidation using periodate compounds can be used to provide reactive carbonyl moieties on the polymeric backbone. Additional methods of activating polymers bearing hydroxyl groups will be apparent to those of skill in the art.

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Polymers bearing sulfhydryl groups can be activated using dithiobispyridyl compounds such as, for example, 2,2'-dithiobis(5-nitropyridine), 2,2'-dithiobis(pyridine), *etc.* Additional methods of use in activating sulfhydryl-bearing polymers will be apparent to those of skill in the art.

5   It will be appreciated by those of skill in the art that the above activation reactions are advanced as examples only and that many further alternatives to these schemes exist.

Once the polymeric framework is activated, it can be reacted with at least ten functional groups as well as ancillary groups or spacer groups and mixtures thereof.

10   Alternatively, the activated polymer can be reacted with a combination of functional groups, ancillary groups and/or spacer groups.

B.   *The Functional Group Component*

The functional group component of the polyvalent presenter prepared in accordance with the methods of the present invention includes those groups capable of being

15   attached to a framework component and of being polyvalently presented in a functional manner, *e.g.*, for treating a disease or condition. The functional group components can be the same or different. The language "a plurality of functional group components" or, alternatively, "a plurality of group R^3" is intended to cover more than ten functional group components, wherein each functional group within the plurality is independently the same or

20   different. The functional group can be the same or different within categories of types of functional groups, *e.g.*, one functional group can be a carbohydrate and another functional group can be an antibiotic. The functional groups can also be different within the same type or category of functional groups, *e.g.*, two different carbohydrates can be functional groups. In the case of a "homomeric presenter," the functional groups presented are the same,

25   whereas in the case of a "heteromeric presenter," the functional groups presented are different, *e.g.*, from different categories or different within the same category. For ease of description below and in the claims, the nomenclature R^3_1 to R^3_n will be used to denote various members of a plurality of R^3.

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As used herein, the functional groups of the subject polyvalent presenters have detectable activity apart from the framework. The functional groups also provide their function when attached to the framework components of the polyvalent presenters. This differs from the art-recognized drug delivery systems, *e.g.*, polymeric drug delivery systems, which release their therapeutic agents and wherein the therapeutic agents provide a function in released form. For example, in preferred embodiments of the present invention, the functional groups directly provide a therapeutic effect by biospecifically interacting with a plurality of target binding sites. In other embodiments, when the polyvalent presenter is, for example, a heteromeric presenter, certain of the functional groups (*e.g.*, R^3_n) may not interact with the target binding sites, but instead provide another function. For instance, the functional groups may influence the interaction of R^3_1 with a corresponding binding site and thereby function as an enhancer group. In other embodiments, the functional groups can provide function by allowing for tracking of the subject presenters, for example, by providing a label which can be detected (*e.g.*, a fluorescent or radioactive tag).

The functional group components of the present invention can be either synthetic or natural. In addition, the functional group components can be categorized based on physical characteristics such as molecular size; the functional group can be of low, medium or high molecular weight. Examples of natural functional group components include, but are not limited to, naturally occurring sugars, proteins (*e.g.*, IgE or erythropoietin), peptides and other known drugs. Examples of synthetic functional group components include, but are not limited to, peptide mimetics, functional groups which are synthesized by combinatorial chemistry techniques or rational drug design techniques. Specific types of functional group components which can be used in accordance with the methods of the present invention are discussed in detail below under the heading "*Types of Functional Groups*".

1.   *Number of Functional Groups Per Polyvalent Presenter*

Various physical properties of the framework component can influence the ability of polyvalent presenters to modulate interactions between the functional group component, R^3, and target binding sites. By varying the number of group equivalents, the

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effectiveness of polymeric presenters can be altered, *i.e.*, can be made to either increase or decrease. For example, it has been found that large numbers of attachment points can cause the presenter backbone to collapse onto the surface on which binding sites are displayed and become less effective in steric stabilization. Moreover, a systematic study of the effects of

5   substituents on a parent polyacrylamide polymer with 0.2 equivalents of sialic acid (SA) indicated that the net charge, size and hydrophobicity of backbone substituents can effect the success of inhibition by polymers (Mammen, *et al.*, 1995, *J. Med. Chem.*, 38:4179). In general, changing the nature of the backbone substituents will affect both affinity and steric stabilization. The term "steric stabilization" refers to a mechanism by which a polyvalent

10   presenter sterically inhibits the close approach of two surfaces by binding to one of the two surfaces. In some cases, the effectiveness of polymeric inhibitors decreases with increasing charge and size of substituents. In such cases, coulombic and steric interactions can cause the chains to become more extended and less effective in steric stabilization. However, it will be readily appreciated that those of skill in the art will know how to vary such

15   parameters to find those which are optimal. Moreover, the combinatorial methods of the present invention allow one to vary the various parameters to identify polyvalent presenters having optimal characteristics, properties and functions.

   2.   *Heteromeric Presenters Having Multiple, Different Functional*
         Groups

20   In addition to the use of different functional group components which impart unique pharmacologic properties to the subject presenters, the use of multiple, different functional groups can offer a number of different advantages. For instance, the use of multiple, different functional groups can offer protection against a range of different pathogens. For example, a polyvalent presenter may present R^3_1 that interact with receptors

25   on the surface of one type of pathogen and, in addition, R^3_2 that interact with a second type of pathogen. Alternatively, different functional groups of a polyvalent presenter may interact in different ways with the same pathogen.

   Heteromeric presentation can also be used to provide both greater *strength* and *specificity* than equivalent monovalent interactions. For example, by presenting an

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additional type of functional group (*e.g.*, R^3_2), the total number of interactions can be increased and the total *strength* of the interactions can also increase. The *specificity* of the interaction can be increased by differentially regulating the number of groups of R^3_1 and R^3_2 which are presented. For example, a presenter bearing both R^3_1 and R^3_2 may interact more tightly with B_1 and B_2 (on a pathogen or on different pathogens) than a presenter bearing either R^3_1 or R^3_2 alone.

Those of skill in the art will readily appreciate that the use of different functional groups, *i.e.*, the use of heteromeric polyvalent presenters, offers numerous advantages other than those set forth above.

3. Types of Functional Group Components

A wide variety of functional groups can be used as the functional group component, *i.e.*, R^3, in Formula I. The functional groups of the present invention include groups useful for treating a disease or condition when presented in a polyvalent manner. Such groups can be known groups or drugs or, alternatively, they can be novel groups or drugs selected after studying a polyvalent interaction involved in a particular disease or condition. The present invention provides for the use of known functional group components, such as those described by Kiessling, *et al.* (*Chem. & Biol.*, 1996, *3*, 71-77) and those identified through the use of combinatorial methods of developing new groups and of enhancing the effectiveness of known monomeric groups by presentation in polyvalent form.

In certain embodiments, functional group components known to be involved in cell-pathogen interaction, cell-cell interaction, pathogen-extracellular matrix interaction, cell-extracellular matrix interaction and pathogen-pathogen interaction can be presented in a polyvalent manner on the subject presenters.

One exemplary group is N-Acetyl Neuraminic acid (a sialic acid), which is the natural binding ligand for influenza hemagglutinin receptor. The interaction between this sugar and its lectin is the first essential step in influenza viral attachment to its target cell. A very large number of polyvalent sugars are possible functional group components, including, but not limited to, Neu5Ac(2,6)Galactose, Neu5Ac(2,6)Lactose,

NeuAc(a2,3)Gal(b1,3)GalNAc, heparin sulfate and galatosyl ceramide, all of which have been shown to be important in the attachment of different viral particles to host cells.

In other embodiments, functional group components known to modulate other diseases or conditions involving polyvalency can be employed on the subject presenters. For example, the functional group components can be selected for their ability to mediate the polyvalent interactions between platelets in situations where it is desirable to treat thrombosis.

In preferred embodiments, the functional group components can comprise known drugs or compounds which have been shown not to be significantly effective in treating a disease or condition when administered in their monovalent form. In these embodiments, the drug becomes significantly more effective when presented polyvalently. Therefore, the present invention provides for numerous, existing drugs that can be incorporated into the subject presenters as the functional group component. It will be understood by one of ordinary skill in the art that the functional group component can have more than one biological effect and/or can be useful in the treatment of more than one type of disease or condition. It should also be understood that some functional group components will be useful for treating diseases or states involving a polyvalent interaction and/or some groups will be useful for treating diseases or states not previously identified as involving polyvalent interactions. The term "drug" is used hereinbelow to refer to possible functional group components for ease of discussion.

For example, drugs which have effects in the central nervous system are provided for as the functional group component. The treatment of Alzheimer's disease can employ, *e.g.*, tacrine or donepezil as the functional group component. In another embodiment, treatment of alcohol dependence can employ disulfiram as the functional group component. Treatment of acute and/or chronic pain and/or inflammation can employ analgesics as the functional group component (*e.g.*, acetaminophen, aspirin, ibuprofen, naproxen, pentazocine, indomethacin, or diflunisal), or anesthetics (*e.g.*, ropivacaine or remifentanil). Treatment of pain and/or narcotic dependence can employ, *e.g.*, hydrocodone, propoxyphenemeperidine, hydromorphone, morphine, methadone or oxycodone as the functional group component. The use of anesthetics is also provided for as the functional

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group component and include, but are not limited to, epinephrine, xylocaine, mepivacaine, methohexital, bupivacaine, novocaine. The subject polyvalent presenters can also use cholinesterase inhibitors, such as pyridostigmine and neostigmine, as the functional group component. Hypnotics can also be incorporated into the subject presenters. Suitable

5   hypnotics include, but are not limited to, fluazepam, pentobarbital, triazolam, temazepam and secobarbital. The functional group component can also be antitussives including, for example, pseudoephedrine or codeine. The present invention also provides for the use of anti-migraines as the functional group component, the anti-migraines including, but are not limited to, ergot derivatives (e.g., ergotamine or methysergide), sometheptene, serotonin (5-

10   HT) antagonists (e.g., sumitriptan), etc. The functional group component can also be a motion sickness remedy, such as meclizine or scopalamine.

In other applications, the functional group component can also be muscle relaxants including, but not limited to, pyridostigmine, neostigmine, succinylcholine, mivacurium, doxacurium, rocuronium, vecuronium, dantrolene, cyclobenzaprine, baclofen,

15   chlorzoxazone, methocarbamol and papaverine. Nausea can be treated with the use of, for example, prochlorperazine, chlorpromazine, trimethobenzaminde, perphenazine, hydroxyzine or ondansetron as the functional group component. Parasympatholytics can also be used as the functional group component, the parasympatholytics including, but not limited to, biperiden, phenobarbital, ergotamine, dicyclomine, hyoscyamine, glycopyrrolate. Similarly,

20   the parasympathomimetics, such as tacrine, pilocarpine, bethaneechol, edrophonium and yohimbine, can be used as the functional group component. The functional group component can also comprise parinsonism drugs, such as trihexyphenidyl, bentropine, procyclidine, levodopa, bromocriptine, carbidopa and amantadine. The invention also provides for the use of psychotropics as the functional group component in the subject presenters. Similarly,

25   antianxiety agents (e.g., lorazepam, buspirone, chlordiazepoxide, meprobamate, clorazepate, diazepam, alprazolam) can be incorporated in the subject presenters as the functional group component. Antidepressants, such as phenelzine, tranylcypromine, parozetine, fluoxetine, sertraline, amitriptyline, nortriptyline, imipramine and protriptyline, can be used as the functional group component. Antipsychotics (e.g., clozapine, prochlorperazine, haloperidol,

30   loxipine, thioridazine, fluphenazine, risperidone, mesoridazine, trifluoperazine, olanzapine

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and chlorpromazine) can also be used as the functional group component. The use of

psychostimulants (*e.g.*, pemoline or methylphenidate) as the functional group component is

also provided for. Sedatives (*e.g.*, mephobarbital, secobarbital, or temazepam) can also be

used as the functional group component. Seizure disorders can be treated by polyvalent

5   presenters bearing, *e.g.*, felbatol, gabapentin, phenytoin, mephenytoin, ethotoin, lamotrigine,

methsuximide, phensuximideethosuximide, cabamozepine, phenacemide or cabamazepine as

the functional group component. Sympatholytics, such as phentolamine, can also be used as

the functional group component. The functional group component can also include, for

example, anticonvulsants (*e.g.*, fosphenytoin) or antidepressants (*e.g.*, mirtazapine), or drugs

10   that can be used to treat multiple sclerosis (*e.g.*, glatiramer) or epilepsy (*e.g.*, topiranate).

The use of the functional group components with effects in the cardiovascular

system is also provided for. For example, adrenergic agents, such as doxazosin, terazosin,

prazosin, methyldopate, clonidine and labetalol, can be used as the functional group

component. In another embodiment, angiotensin converting enzyme inhibitors, such as

15   captopril, lisinopril, tradolapril and enalapril, can also be used as the functional group

component. Polyvalent presenters can also be made to present angiotensin II receptor

antagonists, such as losartan or valsartan. The use of antiarrhythmics as the functional group

component is also provided for, the antiarrhythmics including, but not limited to,

disopyramide, procainamide, quinidine, propafenone, flecainide, tocainide, propanolol,

20   sotalol, amiodarone and digoxin. In other embodiments, β-Blockers, such as timolol,

metoprolol and atenolol, can be used as the functional group component. In a further

embodiment, calcium channel blockers can be employed as the functional group component.

Examples of suitable calcium channel blockers include, but are not limited to, nifedipine,

nicardipine, diltiazem, felodipine, and verapamil.

25   Diuretics (*e.g.*, acetazolamide, ethacrynic acid, furosemide, spironolactone,

amiloride, chlorothiazide, hydrochlorothiazide) can also be used as the functional group

component. Vasodilators (*e.g.*, papaverine, hydralazine, amrinone) can also be employed as

the functional group component in the subject presenters. The use of vasopressors (*e.g.*,

metaraminol, phenylephrine and isoproterenol) as the functional group component is

30   possible. In another embodiment, the functional group component can comprise

mecamylamine. The functional group component can be a hypolipidemic, such as clofibrate, gemfibrozil, simvastatin, lovastatin, simvastatin, atorvastatine or niacin. Deep vein thrombosis can be treated by the use of, for example, danaparoid as the functional group component. In a further embodiment, anti-hypertensives, such as midodrine, can be used as the functional group component.

For the treatment of cancer, the functional group component can include, for example, anti-androgens (*e.g.*, leuprolide or flutamide), cytocidal agents (*e.g.*, adriamycin, doxorubicin, taxol, cyclophosphamide, busulfan, cisplatin, α2-interferon) anti-estrogens (*e.g.*, tamoxifen), antimetabolites (*e.g.*, fluorouracil, methotrexate, mercaptopurine, thioguanine), *etc.* The functional group component can also comprise hormones (*e.g.*, medroxyprogesterone, estradiol, leuprolide, megestrol, octreotide or somatostatin). In still other embodiments, the functional group component can include, *e.g.*, irinotecan, gemcitabine, toptecan, nilandrone, or docetaxel.

In other embodiments, the subject presenters can be used in gastrointestinal applications. For example, antispasmodics or anticholinergics can be included as the functional group component (*e.g.*, dicyclomine, hyoscyamine, glycopyrraolate, trihexyphenidyl or atropine). Appetite suppressants, such as dextroamphetamine, benzphetamine, phentermine and ormazindole, can also be used as the functional group component. In other embodiments, the functional group component can also include anti-diarrheal agents (*e.g.*, loperamide, diphenoxylate, octreotide). The subject presenters can also include, *e.g.*, proton pump inhibitors (*e.g.*, lansoprazole or omeprazole) as the functional group component.

Functional group components which modulate the endocrine system can also be used in the polyvalently presenters of the present invention. For example, contraceptives (*e.g.*, ethinodiol, ethinyl estradiol, norethindrone, mestranol, desogestrel, medroxyprogesterone) can be used as the functional group component. Functional group components which modulate diabetes can also be used (*e.g.*, glyburide or chlorpropamide). Anabolics, such as testolactone or stanozolol, can also be incorporated into the subject presenters as the functional group component. Androgens (*e.g.*, methyltestosterone, testosterone or fluoxymesterone) can also be used as the functional group component.

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Antidiuretics (*e.g.*, desmopressin) can also be used as the functional group component. The subject presenters can also display, for example, calcitonins in a polyvalent manner. Estrogens (*e.g.*, diethylstilbesterol) can also be employed as the functional group component. The functional group component can also be glucocorticoids (*e.g.*, triamcinolone,

5   betamethasone, *etc.*). The use of progenstogens, such as norethindrone, ethynodiol, norethindrone, levonorgestrel, ethinylestradiol, as the functional group component is also provided for. In yet other embodiments, thyroid agents (*e.g.*, liothyronine or levothyroxine) or anti-thyroid agents (*e.g.*, methimazole) can be used. In other embodiments, hyperprolactinemic disorders can be treated using, for example, cabergoline as the functional

10   group component. In still other embodiments, diabetes can be treated using, for example, miglitol or insulin lispro as the functional group component. The use of hormone suppressors (*e.g.*, danazol or goserelin) as the functional group component is also provided for. In other embodiments, the functional group component can include oxytocics (*e.g.*, methylergonovine or oxytocin). Prostaglandins, such as mioprostol, alprostadil or

15   dinoprostone, can also be employed as the functional group component.

The subject polyvalent presenters can also be used in dermatological applications. Exemplary functional group components with dermatological effects include, but are not limited to, anti-acne agents (such as, isotretinoin, adapalene or tretinoin). Other functional group components which can be employed for dermatological applications, *e.g.*,

20   for the treatment of pruritus, include, but are not limited to, alclometasone, benzocaine, hydroxyzine, fluticazone, mometasone, fluocinolone, clobestasol and desoximetasone.

The functional group component can also affect clotting. For application in anti-coagulation, for example, the functional group component can comprise the anticoagulant, heparin or low molecular weight heparin or antithrombin III or integrilin. For

25   applications in anti-platelet activity, for example, the functional group component can comprise lamifiban, abciximab and/or ticlopidine.

In other embodiments, the functional group components can be chosen for their ability to affect immunomodulation. For example, the release of histamine from mast cells and basophils involves the polyvalent interaction between an allergen and IgE receptors

30   on the cell surface. In such instances, the functional group component can comprise, *e.g.*,

antihistamines, such as benadryl, loratadine, brompheniramine, periactin, promethazine, terfenadine, fexofenadine, azelastine and/or clemastine. In still another embodiment, the functional group component can comprise mast cell stabilizers, such as lodoxamide and/or cromolyn.

5   Other functional group components which modulate the immune system include, but are not limited to, steroids (*e.g.*, triamcinolone, beclomethazone, cortisone, dexamethasone, prednisolone, methylprednisolone, beclomethasone, or clobetasol), histamine H_2 antagonists (*e.g.*, famotidine, cimetidine, ranitidine), immunosuppressants (*e.g.*, azathioprine, cyclosporin), *etc.* Groups with anti-inflammatory activity, such as sulindac,
10   etodolac, ketoprofen and ketorolac, can also be used as the functional group component. Antihistamines (*e.g.*, fexofenadine or azelastine) can also be employed as the functional group component.

          Functional group components which modulate the respiratory system, such as mucolytics (*e.g.*, guaifenesin), can also be used. Antiinflammatory agents (*e.g.*, cromolyn,
15   flunisolide, beclomethasone, or budesonide) can also be used in the subject presenters as the functional group component. The use of bronchodilators, such as ipratropium, metaproterenol, terbutaline, isoetharine, metaproterenol, albuterol and theophylline, as the functional group component is also provided for. For the treatment of asthma, agents such as zafirlukast or zileuton can be employed as the functional group component.
20   The functional group component can comprise antimicrobial agents, such as quinolones (*e.g.*, ofloxacin, enoxacin, lomefloxacin); sulfonamides (*e.g.*, sulfasalazine or sulfamethoxazole); ciprofloxacin, norfloxacin, polymyxin B, bacitracin, neomycin, oxytetracycline, tobramycin, sulfacetamide, fosfomycin; antivirals (*e.g.*, trifluridine, zidovudine (AZT), zalcitabine (ddV), didanosine (ddI), stavudine (d4T)), reverse
25   transcriptase inhibitors (*e.g.*, sold by Merck and Abbott), protease inhibitors (*e.g.*, saquinavir, indinavir, ritonavir), acyclovir, famciclvir, ribavirin, zidovudine, nevirapine, cidofovir, or penciclovir); antiparasitic agents (*e.g.*, thiabendazole, chloroquine, sulfadoxine, pyrimethamine, mefloquine, metronidazole, albendazole, or ivermectin); or antifungals (*e.g.*, butenafine, butoconazole, terconazole, tioconazole, clotrimazole, or miconazole). In certain
30   instances, it will be obvious to the ordinarily skilled artisan that the use of particular

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antimicrobials is preferred for treating infections at particular sites, *e.g.*, ophthalmic infections.

In other embodiments, the penicillin adjuvant, probenecid, can be employed as the functional group component.

5   Urinary tract agents for use as the functional group component include, but are not limited to, uricosuric agents, *e.g.*, sulfinpyrazone. Antimicrobials, such as indanyl carbenicillin, nitrofurantoin, nalidixic acid, neomycin, bacitracin and polymyxin B, can also be used as the functional group component. The subject presenters can also present antispasmodics (*e.g.*, oxybutynin or flavoxate). Calcium oxalate stone preventatives, such as

10   allopurinol, can also be used as the functional group component. Prostatic hypertrophy modifiers (*e.g.*, terazosin or finasteride) can also be employed as the functional group component. The subject presenters can also be used in the treatment of cystitis using, for example, pentosan as the functional group component.

The subject presenters are also useful in ophthalmic applications. For

15   example, β-Blockers (*e.g.*, brominide, betaxolol, atenalol or timalol), antiinflammatories (*e.g.*, clopatadine) or drugs useful in the treatment of glaucoma (*e.g.*, latanoprost) can be used as the functional group component. The invention also provides for the use of carbonic anhydrase inhibitors, such as dichlorphenamide, methazolamide or dorxolamide, as the functional group component.

20   For dental applications, such as aphthous ulcers, the functional group component can include, for example, amlexanox.

4.   *Approaches for the Identification of Novel Functional Groups*

In addition to the foregoing known functional group components, the present invention provides methods for the discovery of new functional group components within the

25   context of polyvalency. Once identified, the new functional group components can be used in the combinatorial methods of the present invention to generate libraries, *i.e.*, arrays, of polyvalent presenters. Methods which can be used to identify new functional group components are known to and used by those of skill in the art.

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For example, a single phage in a phage-display library, single pins in a spatially addressed combinatorial array, or single beads in a combinatorial library produced by the mix-and-split method, each bear only one type of group which is presented in polyvalent form. Thus, libraries can be screened directly in bound form against polyvalently

5   presented binding sites for activities such as simple adhesion, or adhesion which leads to a specific result, such as infection, cell death, cell proliferation, morphological change or the production of an easily detectable reporter, such as green fluorescent protein. A typical resin bead can have a loading of ~100 pmol or about 60 trillion copies. Not all of these copies will reside at the surface of all solid supports, but supports can be chosen for, *e.g.*, porosity or

10   surface functionalization. In contrast, phage can display 3-1000 copies of peptide groups on the surface, depending on the coat protein to which the library is fused. Any species of functional group identified as a candidate worthy of further analysis could be subsequently tested in soluble, polymeric form.

By way of example, beads in a library to which bacterial, fungal or neutrophil

15   cells specifically adhere carry groups that may serve as useful groups for a polyvalent presenter of the present invention. Libraries of ~ 1,000,000 different peptides can be prepared by split synthesis, and diverse libraries of other types of compounds may be obtained. The structures of selected groups can be determined from the synthesis history or can be obtained by sequencing, mass spectrometry, deconvolution, encoding, *etc.*

20   For example, phage-displayed peptide libraries can be used to identify new functional group components for use in the methods of the present invention (*See, e.g.,* Doyle, M.V., *et al.*, "The Utilization of Platelets and Whole Cells for the Selection of Peptide Ligands from Phage Display Libraries" in *Combinatorial Libraries: Synthesis, Screening, and Application Potential* (Cortese, R., Ed.; Walter de Gruyter: Berlin; 1996; pp. 159-174);

25   Fong, *et al.*, *Drug Dev. Res.*, 1994, 33, 64-70; Goodson, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 1994, *91*, 7129-7133). In one example, PIII-modified phage (3-5 copies per particle) can be selected ("panned") for interaction with whole, activated platelets, leading to the identification of five different classes of platelet-binding peptide groups. Synthetic peptides derived from phage sequences can be tested for inhibition of phage-platelet interactions and

30   prevention of platelet aggregation, although the observed IC_{50} values were high.

Combinatorial synthesis of polysaccharides using highly efficient glycosyl

donors has also been described (*see*, Liang, *et al.*, *Science*, 1996, 274:1520). Such a strategy

depends on anomeric sulfoxides as glycosyl donors, which are highly reactive and allow

synthesis of polysaccharides in very high yield, which is an essential characteristic of

5   combinatorial libraries in general. Libraries can be created on beads and screened for

interaction to polyvalent lectins in solution. Adhesion assays or agglutination assays can be

used in this system.

Techniques of rational drug design, including the use of computer-based

design tools, (Jorgensen, W. L., "A new method for predicting binding affinity in

10   computer-aided drug design," *Chemtracts: Org. Chem.*, 1995, *8*, 374-376) can also be used to

identify novel functional groups, R^3, for polyvalent presentation.

In addition, the present invention provides for the synthesis of oligosaccharide

groups for use as the functional group component (*see*, Horton, D. Advances in Carbohydrate

Chemistry and Biochemistry; Academic Press: San Diego, 1995). Protein analogs can be

15   synthesized (Maassen, *et al.*, *Eur. J. Biochem*, 1981, 115, 153-158; Wang, *et al.*, *J. Virol.*,

1992, 66, 4992-5001; Mastromarino, *et al.*, *J. Gen. Virol.*, 1987, 68, 2359-2369). Computer

based design tools can be also used to synthesize R^3 groups (Jorgensen, W. L., *Chemtracts:*

Org. Chem., 1995, *8*, 374-376).

20   **5.   *Attachment of the Functional Group Component***

As described herein, the functional group component can be attached to or

formed as part of a framework using a means which allows for polyvalent presentation of the

groups. The attachment can be direct, *i.e.*, without a spacer group or linker, or, alternatively,

it can be indirect, *i.e.*, with a spacer group or linker. The functional group component also

25   can be attached to monomer units before polymerization or after polymerization. The order

of the attachment steps is not critical to the invention as long as the formed polyvalent

presenter displays the functional groups in a polyvalent manner. For example, the functional

group component can be attached first to a spacer group and then the spacer group-functional

group component moiety can subsequently be attached to a framework component or vice

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versa. Alternatively, the spacer group can be attached to the framework component and then the functional group component can be attached to the spacer group-framework component moiety. The functional group components can also be incorporated into the polyvalent presenters using biofunctionalized monomers bearing functional groups which can then be

5   polymerized. Alternatively, a polymer can be prepared that is reactive, *i.e.*, activated for coupling, and then the functional group components can be caused to react with the polymer (a "preactivation" strategy).

A preferred embodiment involves attaching a spacer group to a functional group component. In an even more preferred embodiment, the spacer group is a moiety

10   independent of the functional group component and the framework component. Spacer groups suitable for use in accordance with the methods of the present invention are described in greater detail hereinbelow (*see*, section D, *infra*). An example of a preferred type of spacer group is a spacer group which leaves a primary amine at the untethered end of the spacer group. The modified group can then be attached to an activated carboxylic acid on the

15   polymer, thereby forming an amide linkage. Amide bonds are relatively stable, occur widely in natural systems, and can be formed cleanly by a range of well-developed methods. Additional advantages can be obtained using a particular spacer group as described herein. For instance, cationic polymers tend to be more antigenic than anionic polymers. The cationic modified ligand can be used in limiting quantities to ensure the consumption of all of

20   the modified groups, leaving only excess carboxylic acid groups on the polymer, which imparts water solubility. As discussed herein, the spacer groups also provide spacing of the functional group component from the framework component. In certain embodiments, the spacer groups also impart flexibility to the polyvalent presenter, *e.g.*, flexible movement of the group. Exemplary spacer groups for use in the subject presenters are described in detail

25   hereinbelow.

The "preactivation" method allows one to construct frameworks, *e.g.*, polymers, with a defined mole fraction of its monomer units presenting different groups. For example, the method can utilize an activated ester of poly(acrylic acid), such as poly(N-acryloylsuccinimide) or pNAS made from polymerization of N-acryloylsuccinimide.

30   The NHS ester of pNAS in DMF can be reacted sequentially with different primary amines

(e.g., R_1NH_2 and R_2NH_2) to form amide bonds. The remaining, unreacted ester groups can be converted to carboxamides or carboxylic acids by treating with excess NH_3 or OH^-, respectively. The distribution of these groups along the polymer backbone is thought to be random using this method. Furthermore, the degree of polymerization (i.e., the total number

5   of monomer units in one polymer) can be held constant as the influence of different side groups on effectiveness is examined. The efficiency of the amide-forming reaction between amines and pNAS can be estimated by use of both ^{1}H-NMR spectroscopy and combustion analysis. This reaction consistently proceeds to > 90% completion.

Another example of direct "copolymerization" is illustrated by the use of a

10   mixture of monomeric derivatives of acrylamides in refluxing THF (see, Spaltenstein, et al., supra). Copolymerization may introduce two major uncontrolled variables in polymerization: (i) unknown differences in rate constants for copolymerization among differently N-substituted acrylamides might result in non-uniform distribution of group along the backbone of the polymer; and (ii) the polydispersity, tacticity and length of the polymer might

15   vary as the sidechain is altered. This can occur owing to the differences in rates involved in the polymerization process (propagation; termination). The "preactivation" method may be preferable where the $pR^3(R^1)$ with uniform structural features are needed.

Alternatively, the combinatorial methods of the present invention, which are illustrated herein and in the appended examples and appendices, can be used to create

20   polyvalent presenters. Briefly, a quasi-solid-state, combinatorial approach can be used to generate libraries of backbone bearing groups poly R^3_1 to poly R^3_n.

In preferred embodiments, polymers will be activated using a process which has a high yield and allows for easy removal of by-products. Further considerations include, but are not limited to, reactivity of by-products with the attached groups, tendency to cause

25   racemization (e.g., with poly(amino acids)), etc. For example, there are a range of chemical methods for activating carboxylic acids for reaction with amines. The relative reactivity of activated carboxylic acids decreases in this order: $RCOHal > (RCO)_2O > RCON_3 > RCO_2R > RCONH_2 > RCOR$, and also increases with the nucleophilicity of the attacking amine. In preferred embodiments an internal anhydride is used.

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With an anhydride or succinimide unit in the polymer backbone, an amide bond is readily formed when reacted with an amine. Intramolecular anhydrides in peptides also can be formed using α-amino-N-carboxylic anhydrides or thiocarboxylic anhydrides.

In certain embodiments mixed anhydrides can be used, *e.g.*, carbodiimides

5   and others. Common approaches to forming mixed anhydrides used in peptide chemistry include using carbodiimides (for example, dicyclohexylcarbodiimide (DCC) or 1-ethyl-3-(3-dimethylamino)propylcarbodiimide (EDC)). EDC may be useful for water-soluble polymers. Other exemplary anhydrides can be formed from reagents such as chloroformates or quinoline derivatives (EEDQ, IIDQ).

10   As mentioned above, in other embodiments, carboxylic acid azides can be used. In the case of the use of carboxylic acid azides, prior reaction is required to form the azide. In other embodiments, imidazoles, such as carbonyldimidazole, can be employed.

In yet other embodiments, compounds, such as p-nitrophenol or N-hydroxysuccinimide, that form activated esters may also be used.

15   In still other embodiments, the polymers used contain hydroxyl groups, and so methods for derivatization of hydroxyl groups can be employed. Such methods are known to the ordinarily skilled artisan.

6. *Other Considerations*

The language "constructing and arranging a plurality of functional group

20   components on a framework component" includes the manipulation of various components of the polyvalent presenter to produce a presenter capable of performing its intended function in the treatment of a disease or condition. The manipulations can be made based upon a viewing of the interaction(s) between the functional group component and the collection of target binding sites in a global manner. The manipulations include, but are not limited to, the

25   positioning, sizing and selection of various components, *e.g.* the framework component, the functional group component, the spacer group and/or the ancillary group of the polyvalent presenter. For example, such language is intended to cover the positioning of the functional group component and the framework component relative to each other or relative to an optional spacer group molecule used to attach the functional group component to the

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framework component, or a backbone spacer group used to connect monomers of the framework component. The language also is intended to include the selection of particular types of framework components, functional group components spacer groups and/or ancillary groups.

5   A framework component can be selected for its ability to form a particular type of "blanket" over a collection of target binding sites when it is part of a polyvalent presenter. For example, some frameworks will form gel-like physical barriers over a collection of binding sites. A framework also can be selected based upon a different characteristic which would be desirable to have in the polyvalent presenter being designed.

10   A framework can be selected based on its "flexibility" and/or its ability to impart flexibility to the polyvalent presenter even after attachment of the functional group component.

           The functional group components can also be selected based on their ability to impart desirable characteristics into the polyvalent presenter. It is important to emphasize that the binding capability of the functional group component is a factor in its selection, but

15   at least part of the present invention is the recognition that a weakly binding the functional group component can be useful in its polyvalent form.

           The positioning of the functional group component on the framework also is intended to be part of the "constructing and arranging" of the polyvalent presenter. The positioning can be made based on the known or predicted (*e.g.*, through the use of molecular

20   modeling of the polyvalent environment) spatial arrangement of a collection of target binding sites. The positioning can be along several different directional axes relative to the framework. For example, the target binding sites may be spaced an average of 10Å apart from their neighboring target binding sites and, therefore, the functional group component can be spaced or positioned along the framework component in the horizontal direction

25   appropriate for providing access to neighboring target binding sites. It should be understood that the functional group components do not have to be positioned to exactly match the distance between neighboring target binding sites, but rather are spaced appropriately to provide access. Other factors besides distance are considered when positioning the functional group component on the framework component, such as the flexibility of the

30   spacer group, the contour of the interface containing the target binding sites, *etc.* For

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example, a flexible linker can make adjustments *in vivo* for providing access to the target

binding sites even if the distance between the functional group component does not exactly

match the distance between the neighboring target binding sites, or if the contour of the

interface is curved rather than flat. The depth of the binding pocket, *e.g.*, 2 Å, also may be

5   known or predictable, allowing for the positioning of the functional group component along

an axis substantially perpendicular to the framework, *e.g.*, a particular length of linker can be

used.

The "constructing and arranging" also is intended to include the selection of a

type and length of spacer group which attaches the functional group component to the

10   framework. The length of the spacer group can be selected based on its ability to present the

functional group component to a target binding site within a binding pocket having a known

or predicted depth and/or diameter. The chemical nature of the spacer group, *e.g.*,

hydrophobicity or hydrophilicity, can also be selected based on knowledge regarding the

environment surrounding the target binding site, *e.g.*, the spacer group may have to pass

15   through a channel or environment known to be hydrophobic or hydrophilic. The spacer

group can also be selected based on its ability to impart a desired property into the polyvalent

presenter, *e.g.*, flexibility. The spacer group and use thereof is described in greater detail

herein below.

The term "blanketing" includes both physical blanketing, *e.g.*, the physical

20   covering up of the target binding sites and steric blanketing, *e.g.*, the steric smothering of

target binding site(s). Examples of physical blanketing include the formation of a gel-like

layer or other type of physical barrier over a collection of binding sites which prevents access

to such sites from groups other than those present on the polyvalent presenter.

The "steric blanketing" of a target binding site is the surrounding of the

25   binding site with the functional group component attached to a framework component in a

velcro-like manner. A functional group component can be bound to the binding site, but

other functional group components can be sterically blocking the same binding site or a

neighboring target binding site. These concepts are generally illustrated in **FIG. 2.** P is a

polyvalent presenter having a polymeric framework 1 that conforms to an interface 3, *e.g.*, a

30   surface containing a collection of target binding sites 5, as it interacts via functional groups A

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with binding sites **B**. Steric occlusion may occur through both framework and unbound functional group components.

The language "collection of target binding sites" includes the binding sites over which a single polyvalent presenter molecule conforms. For example, if a polyvalent presenter has ten functional group components attached to a framework component, then the collection of binding sites would be that span of binding sites over which this molecule conforms, *e.g.* ten to twenty binding sites. It should be noted that not all binding sites have to interact with a functional group component; some sites may be left unoccupied and some may be sterically occluded.

The language "an array of target binding sites" includes more than one collection of target binding sites on an interface. The binding sites within the array do not have to be equally spaced or positioned, but rather can be randomly positioned in various directions along different directional axes relative to the interface, *e.g.*, depending on such factors as the contour of the interface and the clustering configuration of the binding sites. For example, the contour of the interface can be flat or it can be curved.

The language "conformal interface interaction" includes an interaction which occurs while a polyvalent presenter is in a conformed configuration over a collection of target binding sites at an interface, *e.g.*, the molecular region accessible to the functional group component. The polyvalent presenters of the present invention conform to an interface, such as a cell surface, by being flexible enough to adjust to the contour of the interface. This conformation can span greater than about 10 to at least about 10^6 target binding sites.

The language "polyvalent presenter" includes multi-component molecules having greater than 10 functional group components, which are capable of binding to greater than 10 binding sites, and which functional group components are attached to a framework.

The language "polyvalent presentation" or "polyvalent manner" refers to the polyvalent display of a functional group such that the poly(functional group) functions differently than its monovalent equivalent. For example, the poly(functional group) may produce a qualitatively different biological effect than a mono(functional group).

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Groups that are polyvalently presented function collectively as one. Polyvalent presentation or polyvalent manner distinguishes the use of polyvalent presenters from the slow release compounds or polymer systems known in the art. Polyvalent presenters are designed to function polyvalently, and not by releasing pharmacologically active monovalent groups

5   from a carrier or delivery vehicle. In addition, because of the release and diffusion of monovalent groups, slow release compounds act at a site which is different from the site of administration and this does not have to be true in the case of polyvalent presenters.

          C.   _Ancillary Groups_

                  As used herein, an "ancillary group" is a moiety which alters a characteristic

10   of the polyvalent presenter and/or the components making up the polyvalent presenter (*e.g.*, the framework component, the functional group component, the spacer group, *etc.*). Properties which can be modified include, for example, solubility (in water, fats, lipids, biological fluids, *etc.*), hydrophobicity, hydrophilicity, framework flexibility, antigenicity, molecular size, molecular weight, *in vivo* half-life, *in vivo* distribution, biocompatability,

15   immunogenicity, stability, strength of binding to the polyvalent target, *etc.*

                  Those of skill in the art will understand that there is substantial, but not complete, overlap between many of these characteristics and the ancillary groups which will effect changes in these characteristics. For example, it is expected that the introduction of one or more poly(ethylene glycol) (PEG) groups onto the framework of a polyvalent

20   presenter will enhance hydrophilicity and water solubility, increase both molecular weight and molecular size and, depending on the nature of the unPEGylated framework, may increase the *in vivo* retention time. Further, it is expected that PEG will decrease antigenicity and may, through hydrogen bonding to solvent molecules (*e.g.*, water), enhance the overall rigidity of the polymeric presenter. Similar areas of overlap between framework

25   characteristics and ancillary groups capable of affecting these characteristics will be apparent to those of skill in the art.

                  Ancillary groups which enhance the water solubility/hydrophilicity of the polyvalent presenter are useful in practicing the present invention. Thus, it is within the scope of the present invention to use ancillary groups such as, for example, poly(ethylene

glycol), alcohols, polyols (*e.g.*, glycerin, glycerol propoxylate, saccharides, including mono-, oligo- and polysaccharides, *etc.*), carboxylate, polycarboxylates (*e.g.*, polyglutamic acid, polyacrylic acid, *etc.*), amines, polyamines (*e.g.*, polyglycine, poly(ethyleneimine, *etc.*) to enhance the water solubility and/or hydrophilicity of the polyvalent presenter. In preferred

5   embodiments, the ancillary group used to improve water solubility/hydrophilicity will be a poly(ether). In particularly preferred embodiments, the ancillary group will be a poly(ethylene glycol).

         The incorporation of lipophilic ancillary groups, within the structure of the polyvalent presenter, to enhance the lipophilicity and/or hydrophobicity of the presenter is

10   within the scope of the present invention. Lipophilic groups of use in practicing the instant invention include, but are not limited to, aromatic groups and polycyclic aromatic groups. As used herein the term "aromatic groups" incorporates both aromatic hydrocarbons and heterocyclic aromatics. The aromatic groups may be either unsubstituted or substituted with other groups, but are at least substituted with a group which allows their covalent attachment

15   to the polyvalent presenter. Other groups of use in practicing the instant invention include hydrocarbon or fatty acid derivatives which do not form bilayers in aqueous medium.

         In preferred embodiments, the lipophilic ancillary group will be a cyclic group such as a hydrocarbon or heterocycle. In other preferred embodiments, the cyclic group will be a six-membered ring or two or more fused six-membered rings. In particularly preferred

20   embodiments, the ancillary group will be a phenyl or a naphthyl group.

         Also within the scope of the present invention is the use of ancillary groups which result in the polyvalent presenter being incorporated into a vesicle such as a liposome or a micelle. The term "lipid" refers to any fatty acid derivative which is capable of forming a bilayer such that a hydrophobic portion of the lipid material orients toward the bilayer while a hydrophilic portion orients toward the aqueous phase. The lipid will preferably have

25   a critical micellar concentration <100 mM. Hydrophilic characteristics derive from the presence of phosphato, carboxylic, sulfato, amino, sulfhydryl, hydroxyl, nitro, and other like groups. Hydrophobicity could be conferred by the inclusion of groups that include, but are not limited to, long chain saturated and unsaturated aliphatic hydrocarbon groups and such

30   groups substituted by one or more aromatic, cycloaliphatic or heterocyclic group(s).

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Preferred lipids are phosphoglycerides and sphingolipids, representative examples of which
include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine,
phosphatidylinositol, phosphatidic acid, palmitoyloleoyl phosphatidylcholine,
lysophosphatidylcholine, lysophosphatidyl-ethanolamine, dipalmitoylphosphatidylcholine,
dioleoylphosphatidylcholine, distearoyl-phosphatidylcholine or
dilinoleoylphosphatidylcholine could be used. Other compounds lacking in phosphorus,
such as sphingolipid and glycosphingolipid families are also within the group designated as
lipid. Additionally, the amphipathic lipids described above may be mixed with other lipids
including triglycerides and sterols.

The flexibility of the polyvalent presenter framework can be manipulated by
the inclusion of ancillary groups which are bulky and/or rigid. The presence of bulky or rigid
groups can hinder free rotation about bonds in the framework or bonds between the
framework and the ancillary group(s) or bonds between the framework and the functional
groups. Rigid groups can include, for example, those groups whose conformational lability
is restrained by the presence of rings and/or multiple bonds. Other groups that can impart
rigidity include polymeric groups such as oligo- or polyproline chains.

Rigidity can also be imparted electrostatically. Thus, if the ancillary groups
are either all negatively or all positively charged, the similarly charged ancillary groups will
force the presenter framework into a configuration affording the maximum distance between
each of the like charges. The energetic cost of bringing the like-charged groups closer to
each other will tend to hold the framework in a configuration that maintains the separation
between the like-charged ancillary groups. Further, ancillary groups bearing opposite
charges may tend to be attracted to their oppositely charged counterparts and will enter into
both inter- and intramolecular ionic bonds. This non-covalent bonding mechanism will tend
to hold the framework into a conformation which allows bonding between the oppositely
charged groups. The addition of ancillary groups which are charged, or alternatively, bear a
latent charge which is unmasked, following addition to the framework, by deprotection, a
change in pH, oxidation, reduction or other mechanisms known to those of skill in the art, is
within the scope of the present invention.

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Bulky groups can include, for example, large atoms or ions (*e.g.*, iodine, sulfur, metal ions, *etc.*) groups containing large atoms, polycyclic groups, including aromatic groups, non-aromatic groups and structures incorporating one or more carbon-carbon multiple bonds (*i.e.*, alkenes and alkynes). Bulky groups can also include oligomers and polymers which are branched- or straight-chain species. Species which are branched are expected to increase the rigidity of the structure more per unit molecular weight gain than are straight-chain polymers.

In preferred embodiments, rigidity is imparted by the presence of cyclic groups (*e.g.*, cyclic hydrocarbons, heterocycles, *etc.*). In other preferred embodiments, the ring is a six-membered ring. In still further preferred embodiments, the ring is an aromatic group such as, for example, phenyl or naphthyl.

Altering the antigenicity of the polyvalent presenter by judicious choice of ancillary group(s) is within the scope of the present invention. In certain applications, the antigenicity of the polyvalent presenter may need to be decreased. As discussed above, masking groups such as, for example, poly(ethylene glycol) are known in the art to have the capacity to lower the antigenicity of both monovalent and polyvalent compounds. In other applications, it may be desirable to enhance the antigenicity of the polyvalent presenter and thus, elicit an immune response. In these applications, the ancillary group may comprise a group known in the art to enhance the immunogenicity of a hapten. Groups appropriate for enhancing the immunogenicity of a polyvalent presenter include, but are not limited to, proteins such as keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other groups capable of enhancing the antigenicity of a polyvalent presenter will be known to those of skill in the art.

The *in vivo* half-life and *in vivo* distribution are functions of numerous molecular properties including molecular size, molecular weight, charge, hydrophobicity/hydrophilicity, antigenicity, biodegradability and the presence or absence of targeting groups on the polyvalent presenter. The term "targeting groups," as used herein, refers to groups which have an affinity for a cellular receptor. Methods of altering these properties to achieve desired changes in the half-life or distribution of compounds administered *in vivo* are well known in the pharmaceutical and medicinal chemistry arts.

D.   *Spacer Groups*

In another embodiment of the present invention spacer groups or, interchangeably, linker groups are interposed between the framework and the functional group and/or the framework and the ancillary group.

The constructs of this embodiment of the invention have the following general formulae:

$$R^1\{-R^2(-R^3)_m\}_n \tag{II}$$

$$R^1\{-R^2(-R^3)_m\}_n\{-R^4(-R^5)_s\}_t \tag{III}$$

The symbol R^1 in both of these formulae represents the multifunctional framework providing a multitude of attachment sites for spacer groups. Polymers, including dendrimers, polypeptides, polysaccharides and others, are generally useful for this framework. With its multitude of attachment sites, the backbone serves an amplifying function for the functional and/or ancillary groups.

In both of these formulae, the symbols R^2 and R^3 represent a spacer group and a functional group, respectively. The symbol m represents the number of functional groups attached to each spacer, which can be equal to or greater than 1. The symbol n represents the number of spacers, and their associated functional groups, which are attached to the framework, and this will generally be a number in excess of 10.

In Formula III, the symbols R^4 and R^5 represent a spacer group and an ancillary group, respectively. The symbol s represents the number of ancillary groups attached to each spacer. This may be equal to or greater than 1. The symbol t represents the number of spacers, and their associated ancillary groups, which are attached to the framework, and this will generally be a number in excess of 1, preferably well in excess of 1.

In those embodiments represented by Formula II, only the spacer/functional group construct is attached to the framework. In embodiments represent by Formula III, both

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the spacer/functional group and spacer/ancillary group constructs are attached to the framework. In Formula III, the spacers R^2 and R^4 can be either the same group or different and may be present in an approximately 1:1 molar ratio, or a different molar ratio. In the interest of brevity and simplicity, the following discussion is phrased in terms of R^2,

5   however, it should be understood that both R^2 and R^4 are encompassed within this discussion. Correspondingly, the discussion concerning R^3 (functional groups) also encompasses R^5 (ancillary groups).

The spacer R^2 can be any of a wide variety of molecular structures, and will be at least bifunctional to permit attachment to both R^1 and R^3, optionally through linkage

10   groups. Spacers can be those which are stable or can be cleaved *in vivo* by the biological environment. Spacers with multiple binding functionalities at the terminus (*i.e.*, the R^3 end) will accommodate a multitude of functional groups and/or ancillary groups, in which case m will have a value exceeding 1. Spacers of this type serve an amplifying function in a manner similar to that of the framework, although to a lesser extent. Other spacers useful in the

15   invention will have only a single functionality at either end, in which case the value of m will be 1. In addition to the property of *in vivo* cleavability mentioned above, a property of prominent interest is hydrophilicity. Still other useful properties are the ability to lower antigenicity and to increase molecular weight. Spacers with still further properties can be utilized to advantage as well, as will be readily apparent to those skilled in the art.

20   The functional groups represented by R^3 include any group useful to elicit a biological response which can be attached to a spacer. These include known functional groups which have been modified or derivatized in any of a variety of ways to achieve a functional group which will permit attachment to the spacer.

The spacer R^2 may be either a straight-chain or a branched-chain structure.

25   Preferred R^2 groups are those which include a straight chain within their structures, either as the entire spacer group or as the backbone of a branched-chain group. The straight chain may be a chain of carbon atoms or of carbon atoms interrupted with one or more hetero atoms such as oxygen atoms, sulfur atoms or nitrogen atoms. The chain can also be substituted with aromatic groups. The bonds forming the chain may be single bonds, double

30   bonds or triple bonds, although single bonds are preferred. The length of the chain may vary

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widely depending on the desired relationship between the molecular weight of the construct
and the number of functional and/or ancillary groups included on the construct. Best results
will generally be obtained with chain lengths ranging from 4 atoms to 1,000 atoms, with
preferred chains being those of 6 atoms to 100 atoms, and the most preferred being those of

5   from 10 atoms to 50 atoms. The chain as thus described is the backbone of the spacer itself,
and does not include atoms, groups or side chains bonded to the serially bonded atoms
forming the backbone. It does, however, include linking groups at the chain termini joining
the chain to R^1 and R^3, when such linking groups are present.

       In certain embodiments of the invention, the spacer will be hydrophilic in

10   character to impart hydrophilicity to the construct. The spacer may thus be any hydrophilic
group among those known in the art. Examples are polyalkylene glycols, optionally
substituted with groups which may or may not add to their hydrophilic character. Among
polyalkylene glycols, polyethylene glycol is a preferred example. Examples of the optional
substitutions are alkyl groups, alkoxy groups and hydroxy groups. Unsubstituted

15   polyethylene glycol is particularly preferred. The optionally substituted polyalkylene glycol
may vary in length, with the selection of length being based on considerations such as
achieving the desired molecular weight for the construct and imparting the desired degree of
hydrophilic character. In most applications, polyalkylene glycols having molecular weights
ranging from about 100 daltons to about 20,000 daltons will provide the best results, with a

20   range of from about 200 daltons to about 1,000 daltons preferred.

       In embodiments of the invention in which the spacer provides *in vivo*
cleavability to the construct, the spacer may contain any of a variety of groups as part of its
chain that will cleave in a biological fluid at an enhanced rate relative to that of constructs
which lack such groups. Accelerated rates of cleavage enhance the rates of removal of the

25   framework from the functional and/or ancillary groups of the polyvalent presenter. Such
removal can be used to enhance the clearance, reduce the toxicity and improve the
therapeutic efficacy of the polyvalent therapeutic. While the degree of cleavage rate
enhancement is not critical to the invention, preferred examples of these spacers are those in
which at least about 10%, and most preferably at least about 35%, of the cleavable groups are

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cleaved in the biological fluid within 24 hours of administration. Preferred cleavable groups are ester linkages and disulfide linkages.

In further embodiments of the invention, the spacer both imparts a hydrophilic character to the construct and includes a cleavable group as referred to above.

5   Structural formulas for the spacer vary widely. The discussion below is offered only as an example of one type of spacer useful in practicing the instant invention and is not intended to serve as a limitation on the types of spacers of use in practicing the instant invention. One group of structural formulas for spacers which impart a hydrophilic character to the construct are those in which m of Formulae II and III above is 1, and R^2 of

10   Formulae II and III is represented by either of Formulas IV, V or VI below:
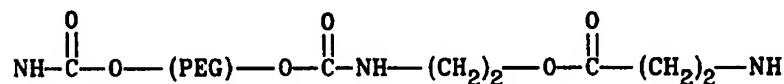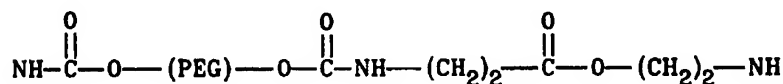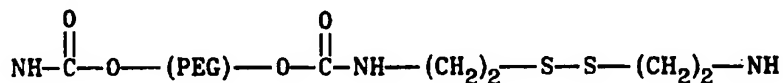
$$X\text{--}R^6\text{--}Y\text{--}R^7\text{--}Z \hspace{3cm} (IV)$$

$$X\text{--}R^7\text{--}Y\text{--}R^6\text{--}Z \hspace{3cm} (V)$$

$$X\text{--}R^6\text{--}Z \hspace{3cm} (VI)$$

In each of these formulas, the hydrophilic component is represented by R^6,

15   which is a polyethylene glycol group having a formula weight of about 100 daltons to about 20,000 daltons, preferably from about 200 daltons to about 1,000 daltons.

In Formulas IV and V, the group R^7 represents a cleavable group which increases the rate of cleavage of the construct in blood. The group is either a disulfide group S--S, or an ester group oriented in either direction, $i.e.$, C(O)--O or O--C(O). Upon cleavage of

20   constructs in which R^2 is represented by Formula IV, the polyethylene glycol group will remain with the polymeric framework R^1. Conversely, upon cleavage of constructs in which R^2 is represented by Formula V, the polyethylene glycol group will remain with the functional and/or ancillary group.
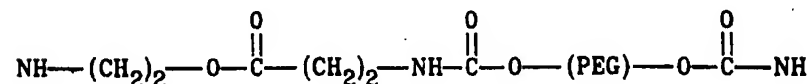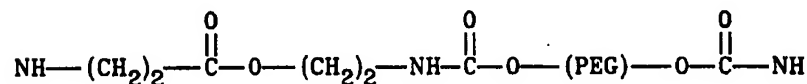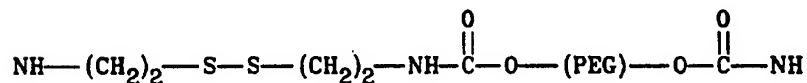
The symbols X, Y and Z represent inert linking groups which serve to join the

25   R-groups together. The nature of these linking groups may not be critical, and their selection will be largely a matter of convenience as determined by the means of synthesis of the

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construct. The term "inert" in this context means essentially non-toxic, non-immunogenic, and stable with respect to cleavage or dissociation over the typical period of time required for use of the construct in a clinical or diagnostic procedure. Examples of inert linking groups useful for this purpose are amides, alkylamino or aminoalkyl groups such as $(CH_2)_q$-NH and NH-$(CH_2)_q$, carbamoyl groups such as NH-C(O)-O and O-C(O)-NH, and alkylcarbamoyl or carbanoylalkyl groups such as $(CH_2)_q$-NH-C(O)-O and O-C(O)-NH-$(CH_2)_q$. The symbol q in these groups may vary, but in most cases will generally range from 1 to 10, with 2 to 4 preferred, and 2 particularly preferred. In the context of this invention, these groups may be defined such that terminal atoms in X or Z may be native to R^1 or R^3, respectively. For example, a terminal NH group in the definition of X or Z may be formed from an amino functional group on R^1 or R^3 or other N-bearing group which can react to form the NH of the linking group. The same is true of a terminal O atom.

Examples of structures defined by Formula IV are as follows:

$$NH-\overset{\overset{\displaystyle O}{\|}}{C}-O—(PEG)—O-\overset{\overset{\displaystyle O}{\|}}{C}-NH—(CH_2)_2—S—S—(CH_2)_2—NH$$

$$NH-\overset{\overset{\displaystyle O}{\|}}{C}-O—(PEG)—O-\overset{\overset{\displaystyle O}{\|}}{C}-NH—(CH_2)_2—\overset{\overset{\displaystyle O}{\|}}{C}-O—(CH_2)_2—NH$$

$$NH-\overset{\overset{\displaystyle O}{\|}}{C}-O—(PEG)—O-\overset{\overset{\displaystyle O}{\|}}{C}-NH—(CH_2)_2—O-\overset{\overset{\displaystyle O}{\|}}{C}—(CH_2)_2—NH$$

Examples of structures defined by Formula V are as follows:

$$NH—(CH_2)_2—S—S—(CH_2)_2—NH-\overset{\overset{\displaystyle O}{\|}}{C}—O—(PEG)—O—\overset{\overset{\displaystyle O}{\|}}{C}—NH$$

$$NH—(CH_2)_2—\overset{\overset{\displaystyle O}{\|}}{C}—O—(CH_2)_2—NH-\overset{\overset{\displaystyle O}{\|}}{C}—O—(PEG)—O—\overset{\overset{\displaystyle O}{\|}}{C}—NH$$

$$NH—(CH_2)_2—O—\overset{\overset{\displaystyle O}{\|}}{C}—(CH_2)_2—NH-\overset{\overset{\displaystyle O}{\|}}{C}—O—(PEG)—O—\overset{\overset{\displaystyle O}{\|}}{C}—NH$$

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In each of the above six structures, the symbol "PEG" refers to a polyethylene glycol segment with terminal hydroxyl groups removed, thereby terminating in ethylene groups at both ends. Spacer groups of a similar general motif which do not contain PEG will also be useful in practicing the instant invention.

5   As a variation of Formula IV, a structural formula for a spacer which supports two or more functional groups is represented by Formula VII:

$$X-R^6-Y'(-R^7-Z)_r \qquad (VII)$$
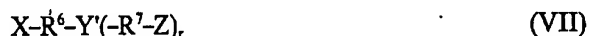
In this formula, R^6, R^7, and X are as defined above, with Z restricted to $(CH_2)_q-NH$. The symbol Y' represents a group of the formula

$$O-C(O)-NH-(CH_2)_q-CH_3 \qquad (VIII)$$

10
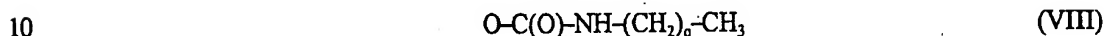
in which q is 1 to 3, and two or more of the H atoms bonded to the C atoms in the $(CH_2)_q-CH_3$ portion of the formula are replaced by the substituent $NH-(CH_2)_s-NH$ where s is 2 to 4, the number of such substituents being equal to m of Formulae II and III such that m is 2 or greater. The result is that the spacer is a branched structure containing two or more

15   reactive NH groups for attachment of functional and/or ancillary groups.

The symbol r of Formula VII is either zero or a number equal to m. When r is zero, the spacer lacks a cleavable group, whereas when r is other than zero, a cleavable group is included for each functional NH group on the Z linker to which a functional group or ancillary group is attached.

20   In preferred examples of Formula VIII, q is 2 to 6, and in most preferred examples q is 2 or 3.

As a further variation, the terminal CH_3 of Formula VIII may be replaced by OH or SH. This results in a reactive OH or SH group available for the attachment of functional and/or ancillary groups.

25   A further group of structural formulas for R^2 of Formulae II and III is that defined by Formula IX:

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$$X'-R^8-Z'$$ (IX)

In Formula IX, R^8 is a group having the formula

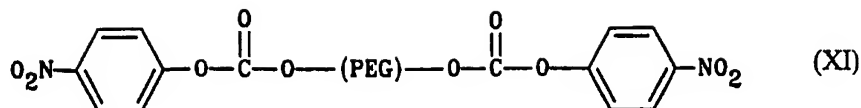$$(CH_2)_o-R^9-(CH_2)_p$$ (X)

in which R^9 a cleavable group bearing the same definition as R^7 of Formulas IV and V above,

5 i.e., either a disulfide group S–S, or an ester group oriented in either direction, i.e., C(O)–O or

O–C(O). The indexes o and p are the same or different and are either zero or a positive

integer, such that the sum of o + p is at least 2.

The symbols X' and Z' in Formula IX are the same or different and are inert

linking groups of scope similar to the inert linking groups of the previous formulas.

10 Preferred examples of X' and Z' are NH–C(O), C(O)–NH, NH–C(S) and C(S)–NH.

In accordance with these various formulas, the number and arrangement of

functional and or ancillary groups on a single construct of either Formula II or Formula III

may vary considerably. The number of functional groups will equal the product of m × n.

The number of ancillary groups will equal the product of s × t. In general, preferred

15 constructs will be those in which either one or both products are at least 10. More preferred

are those in which the product is from 10 to 1,000, and the most preferred are those in which

the product is from 30 to 300.

Constructs in accordance with the present invention may be synthesized in

accordance with conventional linkage reactions which are well known among those skilled in

20 the art. An example, in which the framework R^1 is functionalized with multiple amine

groups, such as polylysine, is offered below. In this example, the backbone is referred to as

$(AMP)(-NH_2)_x$, "AMP" denoting the amplifying effect of permitting the attachment of a

multitude of spacers and functional groups and/or ancillary groups, and x representing a

number corresponding to n in Formulae II and III.

25 The attachment of a polyethylene glycol (PEG) spacer to the amplifier may be

achieved by using an activated ester of PEG, such as an α,ω-bis-p-nitrophenoxy ester of PEG:

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$$O_2N-\text{[benzene ring]}-O-C(=O)-O-(PEG)-O-C(=O)-O-\text{[benzene ring]}-NO_2 \qquad (XI)$$

in which "PEG" is polyethylene glycol minus the terminal hydroxyl groups, as defined

above. An excess of this derivatized PEG ester X is reacted with $(AMP)(-NH_2)_x$ to yield the

intermediate:

5

$$(AMP)\left\{-NH-C(=O)-O-(PEG)-O-C(=O)-O-\text{[benzene ring]}-NO_2\right\}_x \qquad (XII)$$
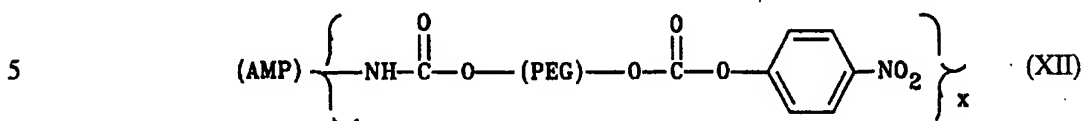
The intermediate is then reacted with an excess of H_2N-(Ligand), which denotes the ligand of

the functional group derivatized to contain a reactive amine group. The product is the

construct:

$$(AMP)\left\{-NH-C(=O)-O-(PEG)-O-C(=O)-NH-(Ligand)\right\}_x \qquad (XIII)$$
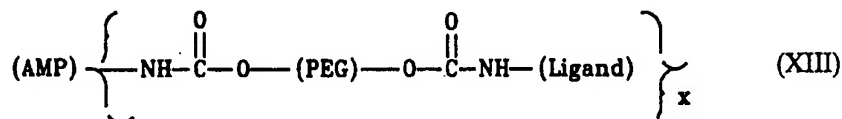
10   In an alternative scheme to a similar product, the above intermediate XI is

converted to a second intermediate with a terminal amine group, by reaction with a diamine

such as $NH_2-(CH_2)_2-NH_2$. The second intermediate has the structure:

$$(AMP)\left\{-NH-C(=O)-O-(PEG)-O-C(=O)-NH-(CH_2)_2-NH_2\right\}_x \qquad (XIV)$$
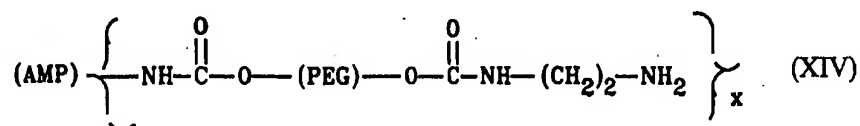
This intermediate XIV is then reacted with a carboxyl-activated ligand, such as, for example,

15   an anhydride of the ligand, to produce a construct having the formula:

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$$\text{(AMP)} \left\{ -NH-\overset{\overset{\displaystyle O}{\|}}{C}-O-\text{(PEG)}-O-\overset{\overset{\displaystyle O}{\|}}{C}-NH-(CH_2)_2-NH-\overset{\overset{\displaystyle O}{\|}}{C}-\text{(Ligand)} \right\}_x \quad \text{(XV)}$$
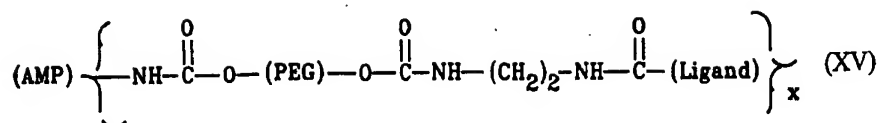
A cleavable group such as disulfide can be introduced by reacting the intermediate X with a diamine containing an internal disulfide, such as cysteamine disulfide, $NH_2-(CH_2)_2-S-S-(CH_2)_2-NH_2$, to yield the further intermediate:

5

$$\text{(AMP)} \left\{ -NH-\overset{\overset{\displaystyle O}{\|}}{C}-O-\text{(PEG)}-O-\overset{\overset{\displaystyle O}{\|}}{C}-NH-(CH_2)_2-S-S-(CH_2)_2-NH_2 \right\}_x \quad \text{(XVI)}$$

This may then be reacted with a carboxyl-activated ligand, to yield:
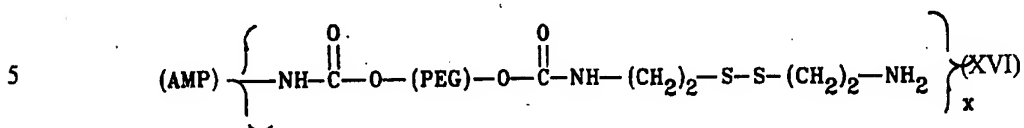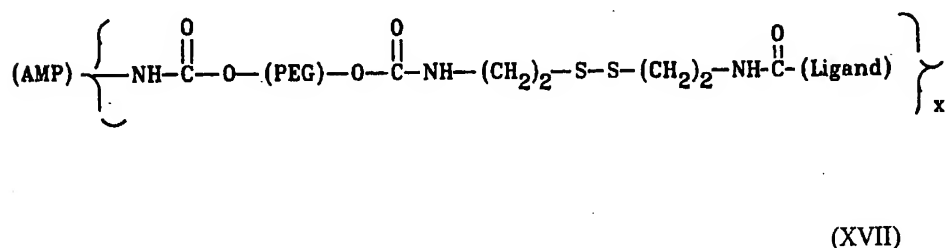
$$\text{(AMP)} \left\{ -NH-\overset{\overset{\displaystyle O}{\|}}{C}-O-\text{(PEG)}-O-\overset{\overset{\displaystyle O}{\|}}{C}-NH-(CH_2)_2-S-S-(CH_2)_2-NH-\overset{\overset{\displaystyle O}{\|}}{C}-\text{(Ligand)} \right\}_x$$

(XVII)

10   Many further alternatives to these schemes exist. To produce constructs containing cleavable esters in the spacers without PEG, for example, an amine- or hydroxyl-containing amplifying polymer can be derivatized to produce carboxylic acid groups as the functional groups. This is readily achieved by reacting the polymer with maleic, succinic or glutaric anhydride using established procedures. A derivatized ligand to combine with the

15   derivatized polymer can be formed by reacting a ligand bearing an isothiocyanate group with an amino alcohol, $HO(CH_2)_nNH_2$, to place a terminal hydroxyl group on the ligand. The carboxylic acid group on the derivatized polymer can then be activated by conventional methods using such agents as dicyclohexylcarbodiimide or carbonyldiimidazole, and reacted with the derivatized ligand to achieve the ester linkage. The section of the construct between

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the amplifying polymer and the ligand serves as the spacer, and the length of the spacer is determined by the number of CH_2 groups in the amino alcohol used to derivatize the ligand.

In an alternate scheme which produces a reverse ester, the ligand is derivatized with an aminocarboxylic acid, $HO_2C(CH_2)_2NH_2$, rather than an amino alcohol.

5   The resulting carboxylic acid-derivatized ligand is then activated with dicyclohexyl-carbodiimide or carbonyldiimidazole and coupled directly to a hydroxyl-containing amplifying polymer.

In either of these two schemes, a selected fraction of the amine or hydroxyl groups which are native to the amplifying polymer can be protected if desired, to avoid

10   interference with the coupling reactions. This is readily achieved by conventional methods, notably acetylation with acetic anhydride.

Ligands with functional groups for attachment to the spacer can be prepared by conventional methods. Well-known ligands for example are readily derivatized by methods known to those skilled in the art. It is preferable to select a ligand which retains all

15   or most of its intrinsic binding affinity even after derivatization.

       E.   *Reaction Variations*

As noted above, the methods of the present invention involve the formation of combinatorial libraries which consist of an array of synthetic polyvalent presenters, wherein the polyvalent presenters in the array differ from one another in terms of their compositions,

20   structures, properties, functions, *etc.* In making the arrays of polyvalent presenters, one can vary, *inter alia*, the chemical structure of the framework component, the chemical structure of the functional group component, the chemical structure of the ancillary group, the chemical structure of the spacer group; the chemical nature of the framework component, the chemical nature of the functional group component, the chemical nature of the ancillary

25   group, the chemical nature of the spacer group; the amount of framework component delivered, the amount of functional group component delivered, the amount of ancillary groups delivered, the amount of spacer group delivered; the number and amount of different framework components delivered, the number and/or amount of different functional group components delivered, the number and/or amount of different ancillary groups delivered, the

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number and/or amount of different spacer groups delivered; the nature and number of the linkages between the various components (*e.g.*, the nature of the linkages of the spacer group); the reaction parameters (*e.g.*, reactant solvents, reaction temperatures, reaction times, reaction initiators, reaction catalysts, the atmospheres in which the reactions are carried out,

5   the rates at which the reactions are quenched, *etc.*); the stoichiometry of the various components; the order in which the different components are delivered, *etc.* The various reactant components (*e.g.*, the framework component, the functional group component, the ancillary group, the spacer group, *etc.*) and the various methodologies which can be employed are described in greater detail hereinbelow.

10   In one embodiment of the present invention, the activated polymer is reacted with one functional group in a manner which consumes substantially all of the activating groups on the polymeric framework. In this embodiment, the functional group to activating group stoichiometry is at least 1:1. An excess of functional group may be added if a 1:1 ratio is insufficient to substantially consume all of the activating groups.

15   In another embodiment of this aspect of the invention, the functional group can be added in an amount insufficient to consume all of the activating groups. Following reaction with the first functional group, either a second functional group or an ancillary group can be delivered and reacted. Also encompassed within this embodiment is the addition of more than one functional group, either as a mixture or sequentially, followed by the addition

20   of an ancillary group or, alternatively, more than one ancillary group as a mixture or sequentially.

In a further embodiment, the activating groups are substantially consumed by the addition of a sufficient amount of a functional group. Following attachment of the first functional group to the framework component, the framework component is reactivated and a

25   second functional group or an ancillary group is added. At either step of the reaction scheme of this embodiment, the functional group(s) may comprise an individual functional group, a mixture of different functional groups, or a mixture of functional groups and ancillary groups, wherein the ancillary groups may comprise either a single ancillary group or a mixture of ancillary groups.

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In yet another embodiment, the activating groups are substantially consumed by the addition of a mixture of at least two functional groups or a mixture of at least one functional group and at least one ancillary group.

In a still further embodiment, less than substantially all of the activating groups are reacted with a mixture of at least one functional group, at least one ancillary group or at least one functional group and at least one ancillary group. Following this first step, the remaining activating groups are substantially consumed by the addition of a further functional group, ancillary group or mixture of functional and ancillary groups.

In certain embodiments, the functional group(s), ancillary group(s) and/or mixtures of the functional and ancillary group(s) are added as dry powders or neat liquids to a solution of the activated framework. In other embodiments, solutions of the functional groups, ancillary groups and/or mixtures of functional and ancillary group(s) are added to a solution of the activated polymer. In still other embodiments, solutions of the functional group(s), ancillary group(s) or mixtures of the functional and ancillary group(s) are added to the polymers. wherein the polymer is present as a neat liquid or a dry powder.

Solvents of use in practicing the instant invention include any solvent is compatible with the presence of the activating groups (*i.e.*, does not react with the activated framework in a significant amount) and the nature of the reaction between the activated polymer and the functional and or ancillary groups. Such solvents include, for example, dimethylsulfoxide (DMSO), dimethylformamide (DMF), alcohols, ethers, ketones, hydrocarbons, aromatic hydrocarbons and mixtures, in any proportions, of these solvents.

In still other embodiments, the temperature can be controlled to control the rate of reaction between the activated framework and the functional group(s) and/or ancillary group(s). In other embodiments, other reaction parameters (*e.g.*, reactant solvents, reaction temperatures, reaction times, reaction initiators, reaction catalysts, the atmosphere in which the reactions are carried out, the pressure at which the reactions are carried out, the rates at which the reactions are quenched, *etc.*) can be varied and optimized.

In yet further embodiments, a "handle" on the functional group or the ancillary groups is activated by means of an activating group as described above and reacted with either an activated group on the framework or an unactivated moiety on the framework.

As used herein "handle" is used to refer to a reactive group such as, for example, carboxyl (acid or salt), hydroxyl, sulfhydryl, amide, carbamate, amine, ketone, aldehyde, olefin, diene, aromatic ring, *etc.* Appropriate combinations of framework-bound reactive moieties and functional group or ancillary group handles will be apparent to those of skill in the art.

5   Those of skill in the art will appreciate that although the foregoing discussion relates to the use of an activated polymer and a functional group, all of the foregoing embodiments are fully applicable to the methods involving the use of a monomer (*e.g.*, an underivatized monomer, a monomer derivatized with a spacer group, a monomer derivatized with an ancillary group either directly or indirectly through a spacer group, *etc.*) and a

10   functional monomer (*e.g.*, a monomer derivatized with a functional group, *i.e.*, R^3, either directly or indirectly through a spacer group).

F.   *Substrate and Delivery Systems*

"Substrate" as used herein refers a material having a rigid or semi-rigid surface or, alternatively, a material having dimples, wells, containers, trenches. *etc.* In many

15   embodiments, at least one surface of the substrate will be substantially flat, although in some embodiments, it may be desirable to physically separate synthesis regions for different materials with, for example, dimples, wells, raised regions, etched trenches, or the like. In some embodiments, the substrate itself contains wells, raised regions, etched trenches, *etc.* which form all or part of the synthesis regions.

20   More particularly, the substrate can be organic, inorganic, biological, nonbiological or a combination of any of these, existing as particles, strands, precipitates, gels, sheets, tubing, spheres, containers, capillaries, pads, slices, films, plates, slides, *etc.* The substrate can have any convenient shape, such a disc, square, sphere, circle, *etc.* The substrate is preferably flat, but may take on a variety of alternative surface configurations.

25   For example, the substrate may contain raised or depressed regions on which the synthesis of diverse polyvalent presenters takes place. The substrate and its surface preferably form a rigid support on which to carry out the reactions described herein. The substrate may be any of a wide variety of materials including, for example, polymers, plastics, pyrex, quartz, resins, silicon, silica or silica-based materials, carbon, metals, inorganic glasses, inorganic

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crystals, membranes, *etc.* Other substrate materials will be readily apparent to those of skill in the art upon review of this disclosure. Surfaces on the solid substrate can be composed of the same materials as the substrate or, alternatively, they can be different, *i.e.*, the substrates can be coated with a different material. Moreover, the substrate surface can contain thereon

5   an adsorbent (for example, cellulose) to which the components of interest are delivered. The most appropriate substrate and substrate-surface materials will depend on the class of materials to be synthesized and the selection in any given case will be readily apparent to those of skill in the art. In a preferred embodiment, suitable substrates include, for example, microtiter plates (*e.g.*, having 96 wells) or a test tube holder containing therein test tubes in

10   an amount sufficient to hold each of the polyvalent presenters of the array.

The reactant components in the individual reaction regions must often be prevented from moving to adjacent reaction regions. Most simply, this can be ensured by leaving a sufficient amount of space between the reaction regions on the substrate so that the various components cannot interdiffuse between reaction regions. Moreover, this can be

15   ensured by providing an appropriate barrier between the various reaction regions on the substrate. In one approach, a mechanical device or physical structure defines the various reaction regions on the substrate. A wall or other physical barrier, for example, can be used to prevent the reactant components in the individual reaction regions from moving to adjacent reaction regions. This wall or physical barrier can be removed after the synthesis is

20   carried out. One of skill in the art will appreciate that, at times, it may be beneficial to remove the wall or physical barrier before screening the array of materials.

In another approach, a hydrophobic material, for example, can be used to coat the region surrounding the individual reaction regions. Such materials prevent aqueous (and certain other polar) solutions from moving to adjacent reaction regions on the substrate. Of

25   course, when non-aqueous or nonpolar solvents are employed, different surface coatings will be required. Moreover, by choosing appropriate materials (*e.g.*, substrate material, hydrophobic coatings, reactant solvents, *etc.*), one can control the contact angle of the droplet with respect to the substrate surface. Large contact angles are desired because the area surrounding the reaction region remains unwetted by the solution within the reaction region.

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In the delivery systems of the present invention, a small, precisely metered amount of each reactant component is delivered into each reaction region. This may be accomplished using a variety of delivery techniques. For instance, the various reactant components can be delivered to the reaction regions of interest from a dispenser in the form

5   of droplets or powder. Conventional micropipetting apparatuses can, for example, be adapted to dispense various droplet volumes from a capillary. The dispenser can also be of the type employed in conventional ink-jet printers. Such ink-jet dispenser systems include, for example, the pulse pressure type dispenser system, the bubble jet type dispenser system and the slit jet type dispenser system. These ink-jet dispenser systems are able to deliver

10   various droplet volumes. Moreover, such dispenser systems can be manual or, alternatively, they can be automated or semi-automated using, for example, robotics techniques.

In other embodiments, the reactant solutions can be delivered from a reservoir to the substrate by an electrophoretic pump. In such a device, a thin capillary connects a reservoir of the reactant with the nozzle of the dispenser. At both ends of the capillary,

15   electrodes are present to provide a potential difference. As is known in the art, the speed at which a chemical species travels in a potential gradient of an electrophoretic medium is governed by a variety of physical properties, including the charge density, size, and shape of the species being transported, as well as the physical and chemical properties of the transport medium itself. Under the proper conditions of potential gradient, capillary dimensions, and

20   transport medium rheology, a hydrodynamic flow will be set up within the capillary. Thus, bulk fluid containing the reactant of interest can be pumped from a reservoir to the substrate. By adjusting the appropriate position of the substrate with respect to the electrophoretic pump nozzle, the reactant solution can be precisely delivered to predefined reaction regions on the substrate.

25   The dispenser of the present invention can be aligned with respect to the appropriate reaction regions by a variety of conventional systems. Such systems, which are widely used in the microelectronic device fabrication and testing arts, can deliver droplets of reactant components to individual reaction regions at rates of up to 5,000 drops per second. The translational (X-Y) accuracy of such systems is well within 1 μm. The position of the

30   dispenser stage of such systems can be calibrated with respect to the position of the substrate

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by a variety of methods known in the art. For example, with only one or two reference points on the substrate surface, a "dead reckoning" method can be provided to locate each reaction region on the substrate. The reference marks in any such systems can be accurately identified by using capacitive, resistive or optical sensors. Alternatively, a "vision" system employing a camera can be employed.

In another embodiment of the present invention, the dispenser can be aligned with respect to the reaction region of interest by a system analogous to that employed in magnetic and optical storage media fields. For example, the reaction region in which the reactant component is to be deposited is identified by its track and sector location on the disk substrate. The dispenser is then moved to the appropriate track while the disk substrate rotates. When the appropriate reaction region is positioned below the dispenser, a droplet of reactant solution is released.

In some embodiments, the reaction regions may be further defined by dimples in the substrate surface. This will be especially advantageous when a head or other sensing device must contact or glide along the substrate surface. The dimples can also act as identification marks directing the dispenser to the reaction region of interest.

Those of skill in the art will readily appreciate that the structure, *i.e.*, composition, of polyvalent presenters can be determined from the synthesis history or can be obtained by sequencing, mass spectrometry, deconvolution, encoding, *etc.* Such evaluation strategies are described, for example, by Thompson *et al.*, *Chem. Rev.*, 1996, *96*, 555-600, the teachings of which are incorporated herein by reference.

II.   *PHARMACEUTICAL COMPOSITIONS*

The present invention also pertains to pharmaceutical compositions for polyvalently presenting an agent for therapy. The pharmaceutical compositions contain a polyvalent presenter, as described in detail above, and a pharmaceutically acceptable carrier. In one embodiment, the polyvalent presenter can have a formula as follows:

$$R^1\{-R^2(-R^3)_m\}_n \tag{II}$$

wherein R^1 is a framework, R^2 is a direct bond or a linker, R^3 is a presented functional group, m is an integer having a value equal to one or greater, and n is an integer having a value

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greater than ten and which is selected such that the presented functional groups can interact with a collection of greater than ten target binding sites. The presenter itself can serve as its own pharmaceutically acceptable carrier. In one embodiment, the polyvalent presenter is made, e.g., n is selected to be greater than 10 and the $-(R^2(-R^3))$ moieties are attached to R^1,

5   such that the polyvalent presenter conforms to an interface containing a collection of greater than 10 target binding sites and blankets the collection of target binding sites upon administration to a subject. In another embodiment, R^2 is a linker group which is an independent moiety and is not part of R^1 or R^3 and n is greater than 10 and is an integer selected such that the polyvalent presenter conforms to a collection of greater than 10 target

10 binding sites upon administration to a subject. In yet another embodiment, R^1 is a polymeric framework, R^3 is a presented functional group and R^2 is a linker group and n is greater than ten and is an integer selected such that the polyvalent presenter conforms to a collection of greater than ten target binding sites upon administration to a subject.

The language "pharmaceutically acceptable carrier" is intended to include

15 substances capable of being coadministered with a polyvalent presenter to allow it to perform their intended function. Examples of such carriers include solutions, solvents, dispersion media, delay agents, emulsions and the like. The use of such media for pharmaceutically active substances are well known in the art. Any other conventional carrier suitable for use with the polyvalent presenter(s) also fall within the scope of the present invention.

20   The language "therapeutically effective amount" of the polyvalent presenter is that amount necessary or sufficient to perform its intended function within the subject. The therapeutically effective amount can vary depending on such factors as the type of site being targeted, the type of components (e.g., frameworks, e.g., linkers, e.g., functional groups R^3) employed, the size of the subject, or the severity of the symptom(s). One of ordinary skill in

25 the art would be able to study the aforementioned factors and make the determination regarding the effective amount of the polyvalent presenter without undue experimentation. An in vitro or in vivo assay also can be used to determine an "effective amount" of the polyvalent presenter. The ordinarily skilled artisan would select an appropriate amount of the polyvalent presenter for use in the aforementioned assay.

The data obtained from cell culture assays and animal studies can be used in formulating an appropriate range of dosages for use in subjects. The dosage of such agents lies preferably within a range of circulating or tissue concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any agent used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a concentration range that includes the IC50 (*i.e.*, the concentration of the test modulating agent which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

The regimen of administration also can affect what constitutes an effective amount. The polyvalent presenter can be administered alone or in conjunction with other agent(s). Further, several divided dosages, as well as staggered dosages, can be administered daily or sequentially, or the dose can be continuously infused. Further, the dosages of the polyvalent presenter(s) can be proportionally increased or decreased as indicated by the exigencies of the therapeutic situation.

Pharmaceutical compositions for use in accordance with the present invention may also be formulated in conventional manner using one or more physiologically acceptable carriers or excipients as the pharmaceutically acceptable carrier. Thus, the polyvalent presenters and their physiologically acceptable salts and solvates may be formulated for administration by, for example, topical application, injection, inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

As such, the agents of the invention can be formulated in a manner appropriate for a specific mode of administration chosen, including, *e.g.*, systemic and topical or localized administration. Techniques and formulations generally may be found in Remmington's Pharmaceutical Sciences, Meade Publishing Co., Easton, PA. For systemic administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the polyvalent agents of the invention can be formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or

Ringer's solution. In addition, the agents may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included.

For oral administration, the pharmaceutical compositions of polyvalent presenters may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid compositions for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid compositions may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The compositions may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Compositions for oral administration may be suitably formulated to give controlled release of the active modulating agent.

For administration by inhalation, the compositions for use according to the present invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.* gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the polyvalent agent and a suitable powder base such as lactose or starch.

The agents can be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in

unit dosage form, *e.g.*, in ampules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for

5   constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

     The agents may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

     In addition to the formulations described above, the agents may also be

10   formulated as a depot composition. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the modulating agents may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

15   Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration

20   may be through nasal sprays or using suppositories. For topical administration, the oligomers of the invention are formulated into ointments, salves, gels, or creams as generally known in the art.

     In embodiments wherein the polyvalent presenter is large and does not pass passively and rapidly through hydrophobic membranes, injection or inhalation may be more

25   appropriate than ingestion or transdermal delivery. These issues of delivery are related to those for protein-based drugs.

     The compositions may, if desired, be provided in a pack or dispenser device, or as a kit with instructions. The composition may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil,

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such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration, *e.g.*, for use in the methods described herein.

III.   *METHODS FOR TREATING A DISEASE OR CONDITION*

The present invention also pertains to methods for treating a disease or
5   condition with polyvalent presenters prepared by the combinatorial chemistry methods described herein and selected for useful properties by screening the resultant combinatorial libraries, as described below (**Section IV**). The method involves administering an efficacious amount of a polyvalent presenter to a subject in need of treatment such that the treatment of the disease or condition occurs.

10   The polyvalent presenter that facilitates treatment meets the following criteria:

the groups R^3 are functional and act as a drug, alone, or in combination with the framework;

the presentation of the groups R^3 attached to the framework provide an
15   additional benefit to the interaction relative to the presentation of a single R^3 to a plurality of binding sites; and

the additional benefit is a synergistic benefit in that the benefit is greater than the additive benefit that would have been provided by a collection of monomers of the same R^3 dispersed in a homogenous solution.

20   The additional benefit is selected from the group consisting of the provision of a sufficient biological effect at a lower concentration of groups R_3, the enhancement of specificity for a targeted versus non-targeted site, and the enhancement of biological potency. In a preferred embodiment, the polyvalent presenter provides at least two additional benefits and in an even more preferred embodiment at least three additional
25   benefits. In the embodiment including the provision of three additional benefits, the benefits can be selected from the above list and also from those benefits described below in the sections describing the pharmacodynamics and/or the mechanisms related to the functioning of the polyvalent presenters.

For the methods for treating a disease or condition, the binding sites with which the groups R^3 of the polyvalent presenter interact can be either intracellular or extracellular (*e.g.*, on the cell surface or on extracellular matrix) or localized on cell membranes. The polyvalent presenters of the present invention can have a variety of

5   functions. In terms of facilitation of treatment. They can be used to modulate (*e.g.*, up or downregulate) a state associated with polyvalency, for example, an unwanted state (*e.g.*, fertilization) or a disease state (*e.g.* infection). The subject presenters can be used to target specific biological events of the disease or condition (*e.g.*, prophylactic prevention of cell-pathogen attachment) or can be used to treat specific disease states (*e.g.*, to control and

10   eliminate an existing infection in a patient). Likewise, the subject presenters have a variety of *ex vivo* applications, *e.g.*, materials and systems for passive protection against biological threats, components for systems for decontamination and new diagnostic and characterization systems. In such applications, the polyvalent presenters are designed to block infection or intoxication of a subject.

15

A.   *Modulation of Specific Biological Events*

In certain embodiments, the polyvalent presenters of the present invention can be used, for example, to modulate cell-cell interactions. Numerous biological processes require cell-cell interactions and the subject presenters can either promote or inhibit such

20   interactions. For example, the subject polyvalent presenters can be used to inhibit egg-sperm interactions such that fertilization is inhibited, *e.g.* the acrosomal reaction can be interfered with using a polyvalent presenter. Further, the polyvalent presenters can be used to prevent toxins of, for example, bacterial, mycological or botanical origin, from binding to a cell surface.

25   In another embodiment the subject polyvalent presenters can be used to inhibit another type of cell-cell interaction, *e.g.*, selectin-mediated attachment of leukocytes to endothelial cells. The polyvalent presenters of the present invention can be used to interfere with neutrophil-endothelial cell interaction and, thus, modulate states associated with neutrophil adhesion, such as, *e.g.*, inflammation, adult respiratory distress syndrome,

30   rheumatoid arthritis, septic shock, and reperfusion injury.

In another embodiment the subject polyvalent presenters can be used to inhibit platelet-platelet interaction. Platelet aggregation plays a salient role in thrombotic or thromboembolic events. Groups R^3 can be chosen to inhibit any of the interactions leading to platelet aggregation. In a preferred embodiment, the interaction of GPIIb-IIIa and a fibrinogen recognition motif is modulated. In certain embodiments it will be desirable to induce hypocoagulation in a subject, while in other embodiments it will be desirable to induce a hypercoagulative state in a subject.

In another embodiment the polyvalent presenters of the instant invention can be used to reduce the ability of cancer cells to metastasize. Metastasis involves the detachment of tumor cells from primary sites, their invasion into neighboring tissues, and their settlement at secondary sites, and the subject polyvalent presenters can act at any of these stages. The tropism of cancer cells for various organs can be mediated by lectins of the invaded organs or of the tumor cells (Matrosovich, *LETT*, 1989, *252*:1,2:1-4; Beuth, *et al.*, 1988, *Clin. Exp. Metastasis*, *6*:115-120). For example, β-lactosyl clusters have been described as potential tumor metastasis inhibitors (Dean, *et al.*, 1993, *Carbohydrate Research*, *245*:175). Accordingly, polyvalent presenters can be made which incorporate modulators of these recognition events as groups R^3.

In another embodiment the subject presenters can be used to modulate infection. The initial step in most cell-pathogen interactions involves attachment. This is true for all viruses. Most bacterial infections are also initiated by an adhesion step involving bacterial adhesions and carbohydrate determinants present on the host cell (Matrosovich, *FEB LETT*, 1989, *252*:1,2:1-4). In the selection of groups R^3 for polyvalent presentation, the use of groups R^3 that interact with the pathogenic particle are preferred over those that interact with the host. In one embodiment, the subject presenters are used to block cell-bacteria interactions. In another embodiment, the subject presenters are used to block cell-fungus interactions. In yet another embodiment, the subject presenters are used to block cell-virus interactions. In a further embodiment, the subject presenters are used to block cell-parasite interactions. For example, the binding of *Entamoeba histolytica* trophozoites to host galactose (Gal) and N-acetylgalactosamine (GalNac) residues. (Adler, *et al.*, 1995, *J.*

Biol. Chem., *270*:5164). In some embodiments, *e.g.*, gene therapy applications, it may be desirable to promote cell-pathogen, *e.g.*, cell-virus, interactions.

In another embodiment, polyvalent presenters of the present invention can be made that modulate pathogen-extracellular matrix interactions. In another embodiment, the
5 subject presenters can be used to modulate cell-extracellular matrix interactions.

In yet another embodiment, polyvalent presenters that modulate pathogen-pathogen interactions can be constructed. Such presenters will be useful in the treatment of, *e.g.*, infectious states that result from the use of indwelling devices, such as prostheses and catheters, or in the disruption of biofilms *ex vivo*.

10 In yet another embodiment, cell-toxin interactions may be modulated. For example, shiga toxin from *Shigella dysenteriae* type 1 binds to cellular glycoproteins or glycolipids having the galabiose disaccharide (Galα1-4Galβ) determinant. Polyvalent presenters of the present invention can be made to present groups R^3 which can block any such cell-toxin interaction. In another example, a polyvalent presenter which reacts with a
15 hemorrhagic toxin, such as that produced by *Croatalus viridis* can be made. In a still further example, polyvalent presenters can be used to prevent the binding of the toxic ricin to cell surfaces.

Ricins are, in some aspects of structure and modes of action, related closely to bacterial toxins (anthrax, cholera, shiga, verotoxin) and other plant-derived cytoxic lectins
20 (ribosomal-inactivating proteins; abrin). These toxins are the objects of current research as the cytotoxic components of immunotoxins in development of anticancer drugs, and have been classified as threats in biological warfare. In one embodiment, the present invention utilizes Gal-presenting polymers to prevent the attachment of ricins to mammalian cells.

In another embodiment the subject presenters can be used to modulate
25 cellular responses that are associated with polyvalency - for example, the production of cytokines by tumor cells and by T cells, mast cell and/or basophil degranulation, lymphocyte selection, and T or B cell apoptosis (Seledtsov, *et al.*, 1995, *Biomed. & Pharmacother*, 1996, 50:170).

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B.   *Modulation of Specific States Associated with Polyvalency*

In addition to targeting any of the specific biological events that are associated with the disease or condition, the skilled artisan could design polyvalent presenters that target specific diseases or conditions or manifestations of those events presented by subjects (see *e.g.*

5   Harrison's Principles of Internal Medicine, Thirteenth Edition, Eds. T.R. Harrison, *et al.*, McGraw-Hill N.Y., NY). For example, polyvalent presenters can be designed to modulate infectious disease, including *e.g.*, inhibiting host-parasite interactions, augmenting immunization or vaccination strategies, and reducing sepsis or septic shock. More specifically, the subject presenters will be useful in the treatment of, *inter alia*: infectious

10   diseases of the upper respiratory tract; infective endocarditis; intraabdominal infections and abscesses; acute infectious diarrheal diseases and bacterial food poisoning; sexually transmitted diseases; pelvic inflammatory disease; urinary tract infections and pyelonephritis; infectious arthritis; osteomyelitis and infections of prosthetic joints; infections of the skin, muscle, and soft tissues; infections in injection drug users; infections from bites, scratches,

15   burns, and environmental organisms; and nosocomial infections. Accordingly, the subject presenters will be useful in the treatment of states caused by Gram-positive organisms (*e.g.*, pneumococcal infections, staphylococcal infections, streptococcal infections, corynebacterial infections, listeria infections, tetanus, botulism clostridial infections, and anthrax), and states caused by Gram-negative bacteria (*e.g.*, meningococcal infections, gonococcal infections,

20   *Moraxella* and *Kingella* infections, *Haemophilus* infections, *Legionaella* infections, pertussis, infections with enteric bacilli, *Pseudomonas* infections, salmonellosis, shigellosis, *Campylobacter* infections, cholera and other vibrioses, brucellosis, tularemia, *Yersinia* infections, bartonellosis; and donovanosis. The subject presenters will also be useful in the treatment of nocardiosis, actinomycosis, mixed anaerobe infections, and mycobacterial

25   infections (*e.g.*, tuberculosis, leprosy, and *Mycobacterium avium* infection). Polyvalent presenters can also be used in the treatment of spirochetal diseases (*e.g.*, syphilis, treponematoses, leptospirosis, relapsing fever, and lyme borreliosis). *Rickettsia, Mycoplasma,* and *Chlamydia* infections will, likewise, benefit from treatment with the subject polyvalent presenters.

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Viral infections will also benefit by treatment with the subject polyvalent

presenters. For example, DNA viruses (*e.g.*, herpes simplex, varicella-zoster, Epstein-Barr,

cytomegalovirus infection, poxvirus infection, parvovirus, and human papillomavirus) and

RNA viruses (*e.g.*, retroviruses, influenza, gastroenteritis, enteroviruses and reoviruses,

5   rubeola, rubella, mumps, rabies, rhabdoviruses, and marburg-like agents, arbovirus

infections, and arenavirus infections) can also be treated.

In other embodiments, the subject presenters can be used to treat fungal

infections, *e.g.*, histoplasmosis, coccidioidomycosis and paracoccidioidomycosis,

blastomycosis, cryptococcosis, candidiasis, aspergillosis, mucormycosis, among others can

10   be treated. In other embodiments, polyvalent presenters can be used to treat protozoal

infections (*e.g.*, amebiasis, malaria and babesiosis, leishmaniasis, trypanosomiasis,

toxoplasma, pneumocyctis cariniii, giardiasis, cryptosporidiosis, and trichomoniasis). In still

other embodiments, the subject presenters can be used to treat helminthic infections (*e.g.*,

trichinosis, tissue nematodes, intestinal nematodes, filariasis, loiasis, onchocerciasis,

15   dracunculiasis, schistosomiasis and other trematode infections, or cestodes). In still other

embodiments, the subject presenters can be used to treat ectoparasite infestations.

In still other embodiments the subject presenters will be useful in modulating

the immune response, both by upregulating and downregulating that response. Accordingly,

the subject polyvalent presenters will be useful both in the treatment of immunodeficiency

20   diseases, (regardless of the underlying cause) as well as in the treatment of autoimmune

disease and resulting immune-mediated injury.. In certain embodiments the subject

presenters will be useful in inhibiting graft rejection.

In other embodiments, the subject polyvalent presenters will also be useful in

the treatment of disorders of coagulation and thrombosis and in anticoagulant, fibrinolytic,

25   and antiplatelet therapy.

In still other embodiments, the subject presenters will also be useful in the

treatment of neoplasia. In preferred embodiments, the subject presenters are used to inhibit

metastasis of primary tumors.

In still further embodiments, the subject presenters will also be useful

30   preventing conception, *e.g.*, by inhibiting the sperm-egg interaction or by inducing the

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acrosomal reaction prior to interaction between sperm and egg. In preferred embodiments, the polyvalent presenters of the present invention may be combined with other methods of contraception.

5   *IV.*   *ASSAYS FOR IDENTIFYING AND TESTING POLYVALENT PRESENTERS*

The present invention also provides for methods of assaying for polyvalent presenters which can be used, *e.g.*, in identifying desirable useful groups R^3 or in testing the efficacy of presenters. Such assays can be either *in vitro* or *in vivo*. In addition, *in vitro* assays can be designed which test for the ability of a polyvalent presenter to modulate the

10   interaction between R^3 and target binding sites, or a biological response that results from the interaction of R^3 with target binding sites (*e.g.*, cell adhesion assays, agglutination assays, platelet aggregation assays, ELISA assays, as well as muscle contractility assays, infectivity assays, growth assays, lymphocyte stimulation assays, and the like).

15   A.   *In vitro Screening*

In certain embodiments, *in vitro* assays can be used to test the ability of a polyvalent presenter to interact with target binding sites or to inhibit the interaction of other ligands with binding sites. For example, assays that test the ability of the R^3 groups of a presenter to interact with binding sites on a polyvalent surface (*e.g.*, panning assays) can be

20   used in screening presenters (Charych, *et al.*, *Chem. & Biol.*, 1996, *3*, 113-120).

In another exemplary embodiment, capillary electrophoresis (CE) can be used. CE is a convenient high resolution analytical technique requiring only femtomoles of material. CE allows separation of mixtures of molecules (ions, small molecules, polymers, proteins, micelles) on the basis of their charge and hydrodynamic drag. By adding R^3 groups

25   to the buffer solution in varying concentration, and by monitoring the influence of this concentration on the mobility of injected binding sites, it is possible to quantitate accurately the binding constant of the group for the binding site. This technique is referred to as affinity capillary electrophoresis (ACE). For example, the affinity of a presenter for a whole virus expressing binding sites can be determined using ACE. It has also been shown that

30   ACE forms the basis of a very efficient library search. CE is also useful, as are gel

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permeation chromatography (GPC) and light scattering, in the analysis of presenters, especially when they carry charge.

Surface Plasmon Resonance Spectroscopy can also be used to study binding events at surfaces (see *e.g.*, Mrksich, *et al.*, *Langmair*, 1995, 4383; Mrksich, *et al.*, *J. Am.*

5   *Chem. Soc.*, 1995, *117*:12009; Sigal, *et al.*, *Anal. Chem.*, 1996, *68*:490).

Model Surfaces Based on Self-Assembled Monolayers (SAMs) can also be used to assay the subject presenter molecules. SAMs of alkanethiolates on gold and silver is another model system for studying adsorption, or other molecular events occurring at interfaces. (See *e.g.*, Mrksich, *et al.*, *Ann. Rev. Biophys. Biomol. Struct.*, 1996, *25*:55;

10   Whitesides, *et al.*, *Self-Assembled Monolayers: Models for Organic Surface Chemistry;* CRC Press: Boca Raton, 1995; Mrksich, *et al.*, *J. Am. Chem.Soc.*, 1995, *117*:12009; Sigal, *et al. Anal. Chem.*, 1996, *68*:490; Lopez, *et al.*, *J. Am. Chem. Soc.*, 1993, *115*:5877).

In another embodiment, agglutination can be tested, for example using synthetic beads mixed with pathogens or cells (equivalent to hemagglutination inhibition

15   assays) for the quantitative analysis of polyvalent molecules. Agglutination is a highly convenient method of probing cell-cell and pathogen-cell contacts and 96-well microtiter plates are an especially suitable format for such an assay. Automation of this assay is also possible. For example, a bead can be constructed that presents a group that interacts specifically with the surface of the pathogen or cell. The construction of such a bead can

20   consider steric access to the group relative to the background. In addition, an appropriate attachment point to the group can be considered and may be based on crystal structures where available. In addition, the importance of eliminating or reducing non-specific interactions through the use of an oligoethyleneglycol background, and the importance of the surface density of the group can also be considered. Alternatively, a target cell (or surrogate

25   target cell) can be used in certain embodiments. For example, influenza virus binds to erythrocytes, and erythrocytes, rather than beads, can be used.

In addition, the relative and absolute concentrations of bead presenting R^3 groups and the surface presenting binding sites which cause the mixture to form a "gel" (*i.e.* to agglutinate) can be determined. Colored beads may be used to aid visualization.

30   Polyvalent materials can be constructed that contain varying mole fractions of the group on

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the bead, or of derivatives of the group, or entirely different groups (in the latter case, inhibition can depend on steric stabilization). In general, the potency of the polyvalent material in the assay will be dependent on the mole fraction of monomeric units of the polymer that are connected to active groups. The mole fraction at which potency is

5   maximum is expected to be system-dependent.

These same polyvalent materials can be modified so that various other groups are incorporated into the available positions on the carrier. The structure and mole fraction of various "auxillary" (e.g., R^3_2- R^3_n) groups may increase the specificity and potency of the molecule. One mechanism whereby such enhancement is possible is by randomly locating

10   hydrophobic pockets on the surface of the pathogen (or cell) using a small mole fraction of short hydrophobic side chains. Important properties of the polymer that are expected to control its potency include: mole fraction of active group, charge, hydrophobicity, persistence length, randomness, physical dimensions, and number of associated water molecules.

15   Inhibition assays can also be used, such as those that measure the extent to which a molecule prevents a biological surface, e.g., a virus, from binding to another biological surface, e.g., a cell. The molecule may do so by binding competitively to the receptor, and prevent binding of the surface bound groups to the same binding site. One exemplary assay is the hemagglutination inhibition (HAI) assay which is described in more

20   detail in the Examples. The HAI assay is based on molecules inhibiting viral agglutination (gel formation) of a solution of erythrocytes. The lower limit of effectiveness that HAI can conveniently measure is ~ 1 nM.

Another exemplary assay is the optical collision (OPTCOL) assay, and is based on the inhibition of attachment upon collision of one erythrocytes and one

25   virus-coated microsphere in the presence of inhibitor. This assay is performed under an inverted microscope using parallel optical tweezers, with each of the tweezers holding one of the two colliding species. The OPTCOL assay allows quantitation of effectiveness of inhibitors that are active at << 1 nM.

Both the HAI and OPTOCOL assays yield a concentration at which the

30   inhibition is half-maximally effective. In the HAI assay, the inhibition constant is K_{HAI}. In

OPTCOL, the inhibition constant is K_{OPTCOL}. When measuring inhibition constants whose values are reliably measurable using each assay, $K_{HAI} = K_{OPTCOL}$. Both these inhibition constants can be referred to as K_i.

OPTOCOL is based on the manipulation of biological particles using parallel dual optical tweezers (Chu, *et al., Acc. Chem. Res.,* 1995, *28,* 461-468). This technique enables study of interactions between, *e.g.,* a single erythrocyte and a single microsphere presenting influenza virus. This technique is especially useful for very tight-binding systems and can also be used for studying the mechanisms of polyvalent inhibition.

Most preferred assays measure the characteristics of the polyvalent group that are important for achieving the desired goal. The assay, therefore, must reflect the ultimate purpose of the polyvalent species.

A number of methods exist for probing, assessing and quantitating polyvalent interactions. Some assays may be direct measures of affinity; from these affinities, one may extract free energies of interaction. Other assays may measure a complex aggregate of characteristics, only one of which is free energy of interaction. These other characteristics may include the extent of hydration, the ability to stabilize a molecule or surface sterically, and/or the ability to crosslink multivalent receptors.

To quantify a binding constant thermodynamically (*i.e.,* to obtain a binding constant), the relative proportions of uncomplexed and complexed group (or binding site) must be measured (directly or indirectly). Depending on the stability of the complex (related to its lifetime), different techniques can be used.

Aggregation assays can be used to measure the ability of a polyvalent group to aggregate a polyvalent binding site (precipitation, gel formation, aggregation). For example, a polyvalent presenter can precipitate polyvalent binding sites on a surface in immunoprecipitation assays. Although the affinity of the polyvalent entity is important in determining the ability of the polyvalent presenter to precipitate, other characteristics can be important. For example, at low concentrations, the presenter may not bind to polyvalent binding sites; at some optimal concentration zone, precipitation occurs; while at higher concentrations, each binding site is bound by a group R^3 and precipitation again does not

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occur. In this example, which is analogous to antibody precipitin reactions, affinity alone does not determine the pattern of precipitation.

B.   *In Vivo Screening*

5   *In vivo* assays can be conducted which measure the therapeutic effect of a polyvalent presenter on a disease or condition in an animal (e.g., protection against infection, inhibition of tumor cell metastasis, effects on blood clotting, and the like). Such assays include, but are not limited to, a measure of the inhibitor to prevent polyvalent interaction. The molecule may, for example, not only slow the rate of infection by blocking attachment

10   to host receptors, but may slow the rate of clearance by blocking the clearance mechanisms.

Table II sets forth numerous assays which can be used to screen the polyvalent presenters of the present invention for useful properties.

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Table II. Various techniques available to quantitate polyvalent interactions.

		Enables measurement of			
	Technique	System in which this technique has been used successfully	Affinity	Inhibition of Surface-Surface interactions (Affinity plus Steric Stabilization)	Kinetics	Comments
5	HAI Assay	1. Inhibitors of the Influenza-Erythrocyte interaction 2. Ab interactions with the surface of wide range of bacteria	no	yes	no	widely used; easily performed; in general, limited to inhibition constants greater than nM
	ELISA Assay	Inhibitors of the Influenza-Erythrocyte interaction	yes	no	no	requires synthetic labeling of the polyvalent species; in general limited to dissociation constants greater than 10-50 nM
	Fluorescence Activated Cell Corter	Ab interactions with the surface of a cell	yes	yes, in principle	no	requires covalent modification of the Ab; based on the separation of subsequent quantitation of bound and unbound forms
10	OPTCOL (Optical Collisions using Dual Optical Tweezers)	Inhibitors of the Influenza-Erythrocyte interactions	no	yes	no	enables measurement of physiologically relevent conditions (collision velocity of cells, relative orientation, and other factors are controlled by the user); measurement involves a single cell and a single microsphere coated with viral particles, and the lower limit of measurable inhibition constants is less than 10^{-14} M.

Enables measurement of					
Technique	System in which this technique has been used successfully	Affinity	Inhibition of Surface-Surface interactions (Affinity plus Steric Stabilization)	Kinetics	Comments
Affinity Capillary Electrophoresis	Dimers of Vancomycin interacting with Dimers of D-Ala-D-Ala	yes	no	no	this technique has much promise, and many extensions to other system are possible and underway
Surface Plasmon Resonance	Ab binding to synthetic surfaces presenting different densities of antigen (DNP, anti-DNP system)	yes	no	yes	requires mg of the polyvalent material (other techniques in this table require 10^2 less in general); other related techniques include Acoustic Plate Mode and Surface Acoustic Wave, and are all based on the detection of small changes in dielectric constant near an interface
Pipette Suction	Cell-Cell	yes, in principle	yes, in principle	no	Based on the quantitation of the energy required to deform complementary cell surfaces during adhesion and separation; may be difficult to perform
Shear Flow	Neutrophil interaction with surfaces derivatized non-covalently with different selectins	yes	yes, in principle	no	based on counting large number of events (stuck, non-stuck) under an optical microscope; probability of adhesion is measured as a function of flow rate of solution past surface
Dissociation under influence of gravity		yes	no	no	time to fall off surface is measured, and correlated with strength of adhesion

Enables measurement of					
Technique	System in which this technique has been used successfully	Affinity	Inhibition of Surface-Surface interactions (Affinity plus Steric Stabilization)	Kinetics	Comments
Optical Microscopy-Counting aggregates	Sperm-Egg interactions, and inhibition of those interactions	yes, in principle	yes	no	based on counting a large number of events (bound, unbound) following agitation; probability of adhesion can be measured as a function of concentration of inhibitor; difficult to perform
Atomic Force Microscopy	Surfaces containing streptavidin interacting with surfaces containing biotin	yes	yes, in principle	no	highly sensitive (a single molecule-molecule interaction can be measured); expensive equipment required; covalent derivatization of small objects required
Light Scattering	None	yes	yes, in principle	no	

V.   MECHANISMS OF ACTION

The mechanisms described below are for discussion purposes and should in no way be construed as limiting to the claimed invention. In preferred embodiments, the subject polyvalent presenters exhibit enhanced affinity for binding sites B over that seen for monovalent R^3 ("mono R^3"). In other preferred embodiments, the subject polyvalent presenters have a greater specificity than mono R^3. By "specificity" it is meant that the nonspecific interaction of polyvalent R^3("poly R^3") with binding sites nonB is reduced compared to that observed with mono R^3. In other preferred embodiments, the subject polyvalent presenters produce a biological effect at a lower concentration than that observed for mono R^3. The polyvalent presenters of the present invention can function by one or more of the mechanisms described below.

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A. *Kinetics and Enhanced Affinity*

In certain embodiments the subject polyvalent presenters function by having extremely low off-rates, such that they are effectively "permanently" bound with regard to the timeframe of a biological event relevant to therapy. This difference in kinetics between poly R^3 and mono R^3, *i.e.*, the lower off-rate for the polyvalent form, is another mechanism by which the subject presenters are different from mono R^3.

Studies of the kinetics for high affinity interactions suggest that the enhancement is mostly due to decreases in the rate of dissociation (k_{off}) of the two polyvalent entities rather than to increases in the rate of association. Binding of anti-DNP Ab to DNP-lys, relative to the binding of the same Ab to the DNP-covered surface of FX174, established that the values of k_{on} for binding to the surface differed by only a factor of 2 (k_{on} (surface) ~ $3.7 \times 10^7 \, M^{-1}s^{-1}$, k_{on} (DNP-lys) ~ $8 \times 10^7 \, M^{-1}s^{-1}$), where the values of k_{off} differed by a factor of 10^4 (k_{off} (surface) ~ $3.3 \times 10^{-4} \, M^{-1}s^{-1}$, k_{off} (DNP-lys) ~ $1.0 s^{-1}$). Since the *rate* of a process is related qualitatively (and, very often quantitatively) to its *thermodynamics*, (Agmon, *et al.*, *Chem. Phys. Lett.*, 1977, *52*, 197-201) then these measurements are intuitively consistent with polyvalency. The thermodynamic cost of the first ligand-receptor interaction between two polyvalent entities is approximately the same as the thermodynamic cost of the analogous monovalent interaction; it is therefore plausible that the rates of association might be similar. Dissociation of species interacting polyvalently requires breaking N ligand-receptor interactions. It is therefore plausible that dissociation occurs more slowly in the polyvalent interaction than in the corresponding monovalent one.

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B.   *Steric Inhibition*

In certain embodiments the polyvalent presenters of the present invention function by "steric inhibition". Steric inhibition is a new strategy in the design of effective pharmaceutical agents, for example, in the case of infectious agents. Polyvalent inhibitors of

5   attachment may be designed that involve any molecule that binds tightly to the surface of the infectious agent, *i.e.*, the polymers that present molecules need not be directly involved in attachment. For example, it is possible to build polymers that prevent the adhesion of influenza virus to erythrocytes by presenting groups that bind to the neuraminidase (NA) on the surface of the virus (Choi, *et al.*, *Chem. & Biol.*, 1996, *3*, 97-104). The NA site is

10   commonly regarded by those of skill in the art as not mediating adhesion, thus the anti-adhesive effect observed with a polymer directed toward it may occur as a result of the attachment of the polymeric gel layer to the viral surface. This effect may be "pure" steric inhibition, *i.e.*, with no entropically enhanced occupancy of the active site of hemagglutinin, the protein that the virus normally uses in adhesion. Thus, polyvalent presentation of a drug

15   may change the original mechanism of action for that drug. There are currently no known examples of such drugs.

The mechanism of steric inhibition is believed to be more related to colloidal stabilization than to receptor-mediated events, although it does depend on receptor-directed specificity to target the polymer to the appropriate binding sites. Bringing two moieties or

20   groups together when one or both is coated by a gel layer is unfavorable both entropically (because the conformational mobility of the water-swollen polymer is decreased on approach to another surface) and enthalpically (because of unfavorable osmotic effects). Polymeric presenters are unique in that they function by this type of mechanism, although there are other, related mechanisms that may appear with liposomes (Kingery-Wood, *et al.*, *J. Am.*

25   *Chem. Soc.*, 1992, *114*, 7303-7305) and dendrimers. (Roy, *et al.*, *J. Chem. Soc., Chem. Commun.*, 1988, 1058-1060; Roy, *et al.*, American Chemical Society: Washington, D. C., 1994).

C.   *Adsorption*

In certain embodiments the subject polyvalent presenters function by mediating the adsorption of binding sites on a surface. By such a mechanism, binding sites *e.g.*, a viral particle, may be effectively removed from solution and cleared by a subject.

VI.   *PHARMACODYNAMICS*

The framework that is chosen and its inherent properties will influence the pharmacodynamics of the polyvalent presenter. Two of the properties that will be considered when designing polyvalent presenters of the present invention are solubility and size.

A.   *Solubility*

In most embodiments the polyvalent presenters of the present invention will be more water soluble than conventional pharmaceutical agents. (*e.g.*, in the mg/ml range or higher). Solubility (and size as described *infra*) can influence the pharmacodynamics of the polyvalent presenter. For example, solubility of the presenters may influence one or more of the related characteristics described below.

For example, solubility can influence the clearance profile of the subject polyvalent presenters. Clearance can be dramatically increased as the solubility of a molecule increases. The kidney tends to filter water soluble molecules more rapidly. Also, the rate of drug clearance is directly proportional to the frequency of drug administration.

The water solubility of the subject presenters can also influence the duration of action of the presenters. In preferred embodiments, the subject presenters have a longer duration of action than does monovalently presented R^3.

In other embodiments the solubility of poly R^3 can influence therapeutic index. As used herein, the term "therapeutic index" refers to the (LD50/ED50) as can be determined by methods well known in the art. The therapeutic index as used herein is meant to be calculated on a per-R^3 basis. The therapeutic index is inversely proportional to the frequency of drug administration. Owing to the lower clearance rates of the subject presenters, poly R^3 will be able to be administered to a subject in at less frequent doses than mono R^3. Moreover, poly R^3 will exhibit a lower concentration variance over time in a

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subject at the site of interest than mono R^3; since the rate of clearance of these drugs can be very slow, they can stay at a more even concentration in the blood or other compartment to which they are administered, *i.e.*, polyvalent drugs will have a reduced difference between maximum and minimum concentration at the site of interest (*e.g.*, lower trough-peak

5   variance). Since polyvalent molecules are large they have the distinct advantage that their lifetimes can be significantly longer than those observed for small molecules. Owing to the slower clearance rates of the subject presenters, poly R^3 will be able to be administered to a subject at less frequent dosing intervals than mono R^3. These longer half-lives are advantageous for a variety of reasons. For example: (i) patient compliance and patient

10   happiness would increase as frequency of drug administration is decreased; (ii) patients can be discharged earlier from hospitals than is currently possible; (iii) drugs can be administered that have lower therapeutic indices than is currently possible.

        The solubility of the subject polyvalent presenters can also influence compartmentalization of polyvalent presenters. Polymeric, polyvalent species having high

15   molecular weight will, in general, not cross biological membranes effectively. This characteristic can, in certain embodiments, make it preferable that they be administered by direct delivery into the compartment of interest. Alternatively, this property means that the polyvalent presenters can be excluded from the undesirable compartments. As examples, intravenous injection accesses the vascular compartment; intrathecal injection accesses the

20   cerebrospinal fluid and the central nervous system; the oral route accesses the gastrointestinal tract; eye drops access the ocular compartment, creams and ointments access the epithelium; catherization accesses the biliary tree and the pancreas and the gall bladder, as well as the cystourethral system, and the vagino-tuboovarian system; and finally inhalation accesses the bronchioalveolar compartment.

25   Molecules may also be designed to be kept out of particular compartments. Examples of areas where this concept may be useful include obstetrics (keeping agents out of the fetal circulation), and agents that are toxic to the kidney (keep agents from being taken up by the kidneys by keeping them in circulation), or to keep presenters out of the central nervous system.

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Polyvalent presenters can also have a tendency to stay localized at a site of interest, with the advantage of reducing systemic toxicity and maximizing local concentrations.

In addition, molecules can be designed that are not confined to a compartment when that property is desirable. For example, in the acute setting, it may be undesirable to have a long-acting agent, but it may be important to have the increased potency that a polyvalent agent can bring. In these applications, polymers may be designed to be of intermediate size, or may comprise cleavable connectors. These molecules will be potent but sufficiently small to be filtered by the kidney and thereby cleared.

Some examples of specific compartments include: the eye (*e.g.*, agonists and antagonists of tearing during surgical procedures or antibiotics), the GI tract (*e.g.*, agonists and antagonists of peristalsis (cholinergic agonists) (cathartic) agonists and antagonists of muscle tone (glucagon) (prior to double contrast barium studies). Other examples include: the CNS, the urogenital system (*e.g.*, kidney, ureter, or bladder, vagina, uterus, fallopian tubes (*e.g.*, contraceptives), and the bronchial tree (*e.g.*, antiasthmatic medication, or cystic fibrosis therapies). Still other examples of compartments include: surface (*e.g.*, skin and mucus membranes for topical applications); the ear canal and middle ear (*e.g.*, antibiotics, antivirals), and the blood (*e.g.*, intravenous injection, as well as transdermal and transmucosal delivery vehicles).

B.   *Size*

The size of the polyvalent presenter can influence the same properties of duration of action, therapeutic index, compartmentalization, and clearance profile as can solubility (as described *supra*).

The term "size" is intended to encompass both molecular size in terms of carbohydrates (*i.e.*, Stokes radius) and molecular weight (kD) in terms of proteins. Polyvalent presenters of molecular size above 60 kD or greater than 50 Å mean hydrodynamic diameter are more likely to be compartmentalized than are molecules of smaller molecular size. In particularly preferred embodiments polyvalent presenters of the present invention are greater than 10kD and 100 Å mean hydrodynamic diameter.

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The size of polyvalent presenter selected may vary with the "state" to be treated. For example, for parenteral applications, low molecular weight compounds (less than about 10,000 MW) will generally be cleared more rapidly. Alternatively, larger compounds which contain cleavable linkages that link units small enough to be cleared when

5   released can be used. Molecules of size greater than that of a 60-70 kDa protein may not be filtered effectively by the kidney, which is important in instances where the polyvalent presenters are to be used in the bloodstream. When used for oral, lung or topical applications, the materials may not need to be cleared or degradable *in vivo*.

For drugs that have essentially a zero clearance because they are too

10   large to be cleared by the kidney, and which are not taken up and cleared by the liver, clearance, when desirable, can be induced by a number of mechanisms. For example, small molecular weight pieces (of a size can easily be cleared by the kidney) can be joined by connectors that hydrolyze at a significant rate in the serum. Alternatively, such low molecular weight pieces can be joined by connectors that are hydrolyzed by agents (*e.g.*,

15   enzymes) naturally present in the plasma. In another embodiment, such small molecular weight pieces can be joined by connectors that can be cleaved by agents (*e.g.*, a second drug, either polyvalent or monovalent) that is taken at the time that clearance of the polyvalent presenter is desirable. An example of such a second agent may be a thiol or a chelating agent, and examples of linkages susceptible to these agents may be disulfides and

20   organometallic links.

In general, high molecular weight species cannot access the blood by oral administration. High molecular weight systems are, nonetheless, useful for other applications. In certain embodiments, they will be made available to the subject by transmembrane permeation (across nasal or pulmonary membranes following administration

25   as aerosols). Some polyvalent species may be taken up through cells in the gut, *e.g.*, by formulation to survive the digestive process or administration as suppositories. Large polyvalent agents, because they will not pass from the lung, gut, or respiratory passages into the systemic circulation, can be advantageous in that they have limited side effects.

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VII.   _ADDITIONAL EMBODIMENTS_

In certain embodiments, it will be desirable to treat a subject with one or more polyvalent presenters and, in addition, with a monovalent inhibitor. Such monovalent inhibitors may or may not interact with the same binding site as does R^3 on the

5   polyvalent presenter. For example, monovalent inhibitors of influenza neuraminidase (NA), a hydrolytic enzyme present on the surface of influenza virus, enhance the ability of polyacrylamide presenting HA inhibitors to prevent hemagglutination (Choi, _et al., Chem. & Bio._ 1996, _3_, 97-104). NA sites on the surface of virus act as secondary binding sites for SA. Adding monovalent inhibitors of NA prevents the secondary binding of SA leading to an

10   increase in the effectiveness of these polymeric inhibitors, probably due to increased steric stabilization. In still other embodiments the subject presenters can be used in conjunction with any other method of treatment.

The following invention is further illustrated by the following examples, which should not be construed as further limiting. The contents of all references,

15   provided manuscripts, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference.

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IX.   *EXAMPLES*

EXAMPLE 1.

This example illustrates the generation and *in situ* evaluation of libraries of

5   derivatives of poly(acrylic acid) presenting sialosides as side chains as polyvalent

inhibitors of influenza-mediated hemagglutination. The example describes a simple, micro-

scale method for generating and evaluating libraries of derivatives of poly(acrylic acid)

(pAA) that present mixtures of side chains that influence their biological activity. The

method relies on one-step conversion of poly(acrylic acid anhydride) (pAAn) to linear

10   polymers presenting multiple R groups as side chains, pAA(R). These derivatized polymers

are prepared by ultrasonication of a suspension of pAAn and various aqueous amines RNH_2

directly in the wells of a microtiter plate. Using this method, derivatives of pAA having *N*-

acetylneuraminic acid (NeuAc-L-NH_2) as a side chain were generated (pAA (NeuAc-L) and

assayed for the ability to inhibit hemagglutination (HAI) of chicken erythrocytes by influenza

15   virus A (X-31); the constant (K_i^{HAI}) describing this inhibition is calculated on the basis of

total NeuAc groups in solution. Co-polymeric pAA(NeuAc-L_n; L_n=different linking groups)

with variable mole fractions of NeuAc-L-NH_2 ($\chi^{NeuAc-L}$ =0.02-0.11) exhibited HAI activities

with K_i^{HAI} values between 26.8 and 0.3 μM. Using combinations of NeuAc-L-NH_2 and one

of 26 different primary amines RNH_2, a variety of ter-polymeric pAA(NeuAc-L; R) ($\chi^{NeuAc-L}$ ~

20   0.05; χ^R ~ 0.06) were generated and assayed. Certain ter-polymers yielded values of K_i^{HAI} that

were lower by a factor of ~10^4 than that of the parent co-polymeric pAA(NeuAc-L). The

most active inhibitor was pAA(NeuAc-L; (L)-3-(2'-napthyl)alanine)) (K_i^{HAI} ~0.5 nM).

Typically, the incorporation of hydrophobic -- especially aromatic -- side groups enhanced

activities. These polymers pAA(NeuAc-L; R)) are potent inhibitors of the adsorption of

25   influenza virus to erythrocytes. They were active with only low to moderate levels of

incorporation of functional groups into the side chains: $\chi^{NeuAc-L}$ ~ 5%; χ^R ~ 6%.

The simple strategy described herein streamlines the generation of

derivatives of pAA and the evaluation of the biological activities of these polymers by

carrying out both synthesis and assay in the wells of microtiter plates. The method allows

30   convenient screening of libraries of polymers presenting multiple combinations of side

chains at controlled mole fractions. Since microtiter plates assays are routine in biology and medical sciences, this method may serve generally for screening and obtaining leads for a range of agglutination interactions and other processes that might be influenced by polyvalent inhibitors.

5   pAA was synthesized having multiple R groups as side chains, pAA(R) by sonicating a suspension (0.12 mg /μL) of poly(acrylic acid anhydride (pAAn) (Jones, J.F., *J. Polymer Sci*, 1958, *33*:15.; Brotherton, *et al.*, *J. Org. Chem.*, 1961, 26:1283) and an aqueous solution of an amine RNH$_2$ (0.1 M) contained in a 250-μL well of a microtiter plate (see Appendix, eq. 1.1 and accompanying results).

10   Solutions of copolymers pAA(NeuAc-L) were prepared by reacting NeuAc-L-NH$_2$ with poly(acrylic acid anhydride)(pAAn) using different numbers of molar equivalents (mol eq.) of NeuAc-L-NH$_2$ to pAAn (see Appendix: Scheme 1 (structures 1-4); eq. 1.2 and accompanying results).   The polymer for which mol eq. of NeuAc-L-NH$_2$ = 0) is homopolymeric pAA obtained from the sonication (hydrolysis) of pAAn alone in PBS buffer

15   (137 mM NaCl, 2.7 mM KCl, 7.7 mM Na$_2$HPO$_4$, 1.5 mM KH$_2$PO$_4$, 0.05% NaN$_3$, pH 12). Co-polymeric pAA(NeuAc-L), for which mol eq. of NeuAc-L-NH$_2$ >0, was generated in microtiter plates with 96 conical-bottom wells as follows: (i) placing 6 mg of pAAn into a well; (ii) soaking the powder with a variable amount (19 - 100 μL) of 0.1 M NeuAc-L-NH$_2$ in PBS buffer, pH 12; (iii) immediately sealing the plate with parafilm® and covering tightly

20   with the plate cover, and (iv) ultrasonicating the plate for 0.5 h in a Fisher ultrasonic bath-type cleaner; the sonication also increased the temperature of water in the bath (and the reactants) slowly up to ~50°C. Each solution of generated co-polymers (pH ~ 3) in a well was neutralized to pH ~7 by adding 60 μL of 1.0 M NaOH, and adjusted to 100 to 200 μL (total volume) with PBS , pH 7, before the HAI assay. The above protocol was easily

25   extended to the preparation of ter-polymers pAA(NeuAc-L; R); here, a three-component mixture (pAAn 6 mg), 50 μL of 0.1 M NeuAc-L-NH$_2$ (Scheme 1, 1 or 3) and 30 μL of 0.2 M RNH$_2$) was sonicated.

           The resulting pAA(R) was characterized by examining ^{1}H-NMR (D$_2$O) spectra of lyophilized reaction mixtures (both as a crude mixture and following dialysis) and

30   by gel permeation chromatography (M$_w$ = 39.5 kDa, polydispersity = 1.91, estimated using

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polysaccharide standards). By comparing the integrated intensity of NMR signals from free RNH_2 before and after sonication, the yields of incorporation of RNH_2 were estimated. The ^{1}H-NMR signals of R from pAA(R) were distinguished readily from those of free, unreacted RNH_2 by their shape (the lines due to polymer-attached species are relatively broad) and by

5   their chemical shift (the δ values of CH_2 or CH groups next to the amide group are shifted downfield). The percent yield of incorporation of RNH_2 as an amide group is on the basis of RNH_2:

$$\text{yield of incorporation (\%)} = \frac{\textit{number of moles of -CONHR}}{\textit{number of moles of used RNH}_2} \times 100$$

10

The average value was ~ 90% (±5) from experiments using five different amines RNH_2 (4-aminobenzoic acid, 6-aminohexanoic acid, N-methylhydroxylamine, (L)-arginine and 1

15   ($NeuAc-L_1-NH_2$)). Because amide formation and hydrolysis of anhydride groups were occurring competitively, the efficiency of the former process was affected by the relative reactivity of each RNH_2, and was also sensitive to both the pH of the aqueous solutions of RNH_2 (optimal pH ~ 7 and 12 for aromatic and aliphatic amines, respectively) and to the number of molar equivalents of RNH_2 to pAAn (optimal mol eq. <0.2).

20   This quasi-solid-phase synthetic method was applied to derivatives of NeuAc having different linking groups L (1-4; $NeuAc-L_n-NH_2$, see Scheme 1) to generate co-polymeric derivatives of pAA presenting NeuAc-L as a side chain (pAA(1) - pAA(4)). $NeuAc-L-NH_2$ (1 and 2) were synthesized following published methods: Sparks, et al., J. Med. Chem., 1993, 36:778; Ogura, et al., Carbohydr. Res., 1986, 158:37; Lees, et al., J. Am.

25   Chem. Soc., 1994, 37:3419. The synthesis of $NeuAc-L-NH_2$ (3 and 4) are shown below. Compounds 1 and 2 were used as readily available α-C- and α-O-sialosides. Compounds 3 and 4 were used because aromatic moieties in the the middle of the linkage can enhance the binding affinity of monomeric NeuAc-L to HA site. Following sonication, the crude solutions of polymers were evaluated immediately for hemagglutination inhibition (HAI)

30   activities using an assay based on chicken erythrocytes and influenza virus A (X-31). (see,

e.g., Mammen, *et al.*, *J. Med. Chem.*, 1995, *38*:4179. Choi, *et al.*, *Chem & Biol.*, 1996, *3*:97.
Lees, *et al.*, *J. Med. Chem.*, 1995, *37*:3419; *Tech. Rep. Ser. W.H.O.* 1953, *64*:1; Kilbourne,
E.D., *Bull. W.H.O.*, 1969, *41*:643.)

Table III (Appendix) gives the values of K_i^{HAI} (the lowest concentration of
NeuAc-L groups from pAA(NeuAc-L) in solution that prevents hemagglutination) at various
molar equivalents of NeuAc-L-NH$_2$. The molar equivalent of NeuAc-L-NH$_2$ is related
directly to the mole fraction of NeuAc-containing side chains in the polymer, $\chi^{NeuAc-L}$. The
mole fraction of R-containing side chains in pAA(R)(χ^R), which is defined below, can be
deduced from an equation including mol eq. and yield of incorporation:

$$\chi^R = \frac{[-CONHR]}{[-COOH] + [-CONHR]} = \frac{number\ of\ moles\ of\ -CONHR}{2*\ \{number\ of\ moles\ of\ anhydride\ groups\ of\ pAAn\}}$$

χ^R (%) = 0.5 (mol eq. of RNH$_2$ × (incorporation yield of RNH$_2$).

The HAI activities of these crude pAA(1) (mol eq. = 0.1 - 0.2) are comparable
to that of purified polyacrylamides presenting 1 (K_i^{HAI} = 0.3 μM at χ^1 = 0.05). Table III also
shows three other derivatives of pAA (pAA(2) - pAA(4)) with HAI activities in the
(sub)micromolar range. The HAI activities of all monomeric sialic acids (1-4) were low
($K_i^{HAI} \geq$ 5mM).

The extension of the above method to generate libraries of ter-
polymers was straightforward. pAA(NeuAc-L; R) presenting both NeuAc-L and one other R
group were synthesized simply by sonicating a three-component mixture of NeuAc-L-NH$_2$,
RNH$_2$ and pAAn. Table III summarizes the values of K_i^{HAI} of pAA (NeuAc-L; R), obtained
from combination of NeuAc-L-NH$_2$ (mol eq. = 0.10 and one of 26 different RNH$_2$ (mol eq. =
0.12). Several pAA(1; R) and pAA(3; R) showed activities enhanced by factors of 100 to
~7000 relative to the parent co-polymeric pAA(1) (mol. eq. of 1= 0.10) in which there is no
R group (note that the HAI assay requires the use of a finite amount of virus, and cannot
measure accurately the effectiveness of inhibitors with K_i^{HAI} <1 nM). The activities were
measured directly from crude pAA(NeuAc-L; R). Several control experiments confirmed

that these ter-polymers (and not pAA(NeuAc-L)) were responsible for the high activities. Co-polymers that did not contain sialic acid groups, pAA(R) were inactive at ~ 15 mM; the mixture of pAA(1) and RNH$_2$ gave the same result as that obtained with pAA(1) alone, and the activities of pAA(1; R) either crude or even after dialysis (MW cutoff ~ 6-8 kDa) were

5   the same within a factor of 2. Typically, the incorporation of derivatives of hydrophobic or aromatic amino acids enhanced the activities greatly. The results are related closely to the previous observation that the incorporation of benzyl or aliphatic amines (as amide side chains) into polyacrylamides presenting 1 (χ^1 ~ 0.2) increased HAI activities.

       The best of these pAA(NeuAc-L; R) belongs to a class of hemagglutination

10   inhibitors that have unusually high activities at relatively modest mole fractions of NeuAc-L (~ 5%) and (~ 6%): each 1% in mole fraction of NeuAc-L or R is equivalent to ~ 6 side chains (per polymer molecule). This finding emphasizes the importance of combinations of side chains in modulating the activities of these polyvalent inhibitors.

15   <u>Synthesis of NeuAc-L$_3$-NH$_2$(3) and NeuAc-L$_4$-NH$_2$(4)</u>
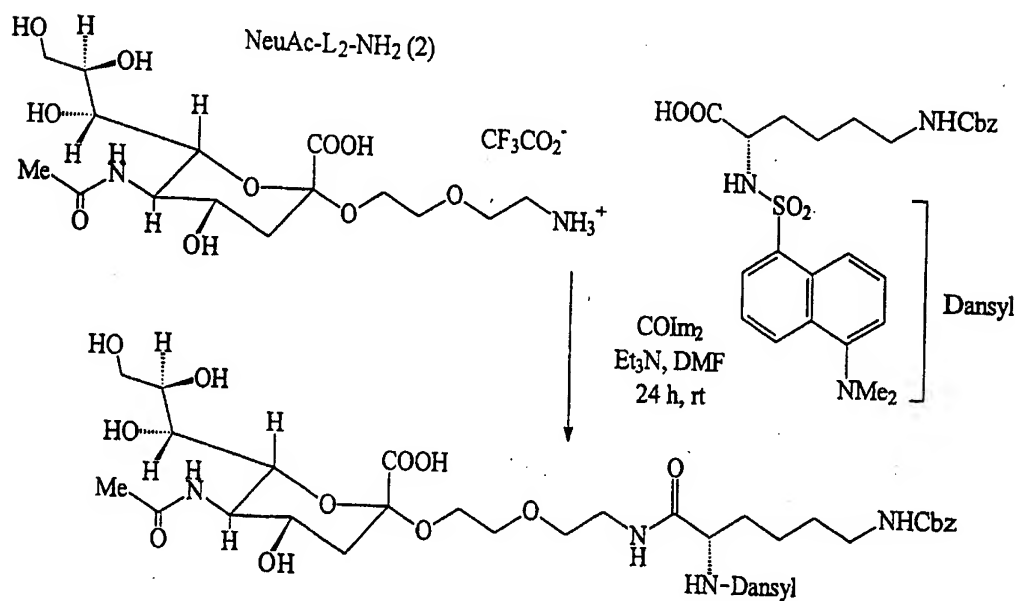
A mixture of carbonyldiimidazole (53 mg, 0.33 mmol) and N^ϵ-Cbz-N^α-dansyllysine (160 mg 0.31 mmol) in DMF (2 mL) was stirred for 2 h at rt, followed by addition of NeuAc-L$_2$-NH$_2$ (2) [NeuAc-L$_2$-NH$_2$ (2) was synthesized according to published methods: Ogura, H. et al. 1986. *Carbohydr. Res.* 158:37; Lees, W.J. et al. 1994. *J. Am.* 5 *Chem. Soc.* 37:3419) (as TFA salt; 120 mg, 0.24 mmol) and Et$_3$N (0.15 mL, 1.08 mmol]. After stirring (24 h at rt), the mixture was concentrated *in vacuo* to yield a pale yellow oily residue. It was dissolved in MeOH (15 mL) containing 5 g of ion exchange resins (Dowex-50W; H+ form). After shaking of the suspension for 5 min. the supernatant solution was separated from resins by filtration through a filter paper and was concentrated prior to flash 10 column chromatography (50 g of silica gel; 10% MeOH/CH$_2$Cl$_2$ to 5% HCOOH/30% MeOH/CH$_2$Cl$_2$). The coupling product was obtained as a pale yellow oil (195 mg, 93%) from fractions with *Rf* of 0.5 (5% HCO$_2$H/30% MeOH/CH$_2$Cl$_2$). ^{1}H-NMR (500.14 MHz, CD$_3$OD): δ (ppm) 8.53-8.50 (m, 1H), 8.38-8.36 (br d, 1H, *J* = 8.6 Hz), 8.21-8.19 (br d, 1H, *J* = 8.6 Hz), 7.57-7.52 (m, 2H), 7.32-7.24 (m, 5H; -COOCH$_2$C$_6$H$_5$), 7.22-7.18 (m, 1H), 5.03 (s, 15 2H;

-COOCH$_2$C$_6$H$_5$), 3.88-3.80 (m, 3H), 3.75-3.48 (m, 9H), 3.27-3.26 (m, 2H), 3.08-3.07 (m, 2H), 2.84 (s, 6H; N(CH$_3$)$_2$), 2.71-2.67 (m, 3H), 2.0 (s, 3H; CH$_3$CONH), 1.63-1.58 (t, 1H, *J* = 12.0 Hz; H$_{3\alpha}$), 1.49-1.48 (br s, 2H), 1.1-1.0 (m, 4H); FAB-MS (glycerol): *m/z* 914 [M+Na]$^-$.

H$_2$ (1 atm), Pd/C (10%)
MeOH, 24 h, rt

NeuAc-L$_3$-NH$_2$(3)
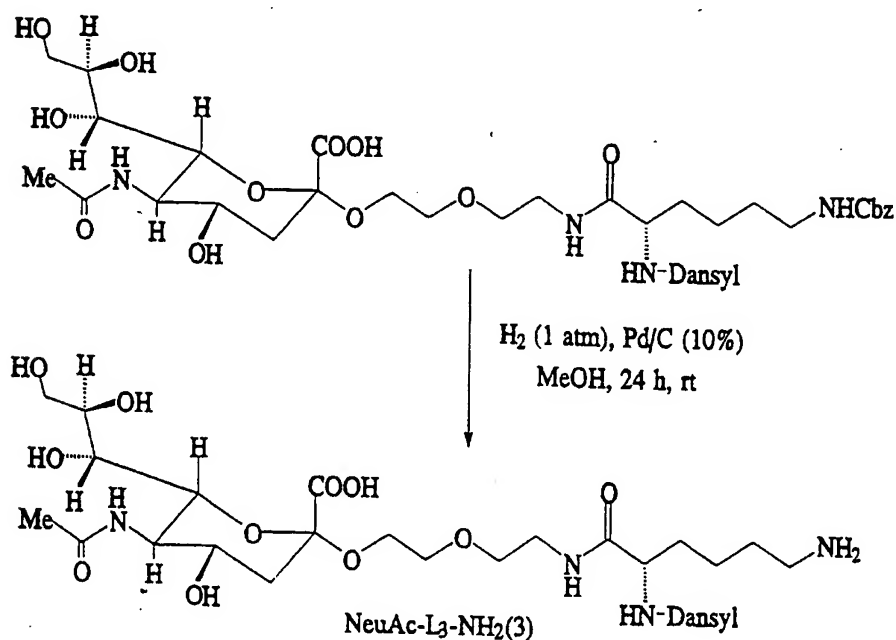
A suspension of N-Cbz protected derivative of sialic acid (180 mg, 0.20 mmol) and 10% Pd/C (100 mg) in methanol (20 mL) was stirred under an atmosphere of H_2 (1 atm) for 24 h at rt. After removal of the palladium catalyst by filtering through a pad of Celite, the filtrate was evaporated *in vacuo* to yield a crude product (NeuAc-L$_3$-NH$_2$ (3)) as a pale yellow oil. The yield of hydrogenolysis was estimated to be quantitative on the basis of ¹H-NMR spectrum that indicates the complete removal of N-Cbz group. ¹H-NMR (399.88 MHz, CD$_3$OD): δ (ppm) 8.56-8.54 (t, 1H, J = 8.69 Hz), 8.38-8.35 (dd, 1H, J=3.27, 8.69 Hz), 8.22-8.20 (d, 1H, J=8.7 Hz), 7.62-7.52 (m, 2H), 7.27-7.23 (m, 1H), 3.91-3.77 (m, 4H), 3.76-3.61 (m, 4H), 3.58-3.56 (m, 4H), 3.48-3.45 (t, 2H , J=4.75 Hz), 3.01-2.98 (m, 2H), 2.86 (s, 6H; N(CH$_3$)($_2$), 2.72-2.62 (m, 3H), 2.0 (s, 3H; CH$_3$CONH), 1.64-1.56 (m, 3H), 1.43-1.40 (m, 2H), 1.18 (m, 2H); FAB-MS (glycerol): m/z 758 [M+H]+ 780 [M+Na]$^+$.
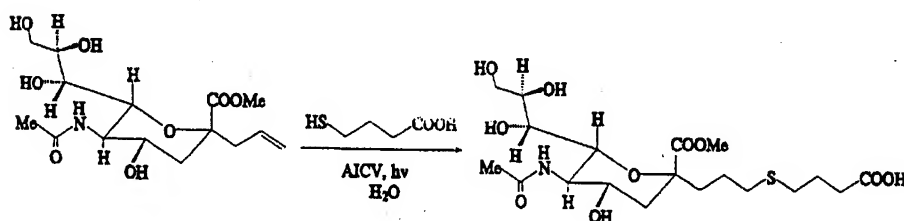

Radical-initiated addition of a thiol to an olefinic group of α-C-allyl sialoside was performed following literature procedures (Roy, R. et al. 1988. *Carbohydr. Res.* 177:C1; Lee, R.T. et al. 1974. *Carbohydr. Res.* 37:193, and Sparks, M.A. et al. 1993. *J. Med. Chem.* 36:778). A suspension of α-C-sialic acid methyl ester (Nagy, J.O. et al. 1991. *Tetrahedron Lett.* 32: 3953 and Paulsen, H. et al. 1991. *Liebigs Ann. Chem.* 487) (0.6 g, 1.73 mmol), 4-mercaptobutanoic acid (Lochon, P. et al. 1976. *Tetrahedron* 32:3023) (0.8 g, 6.67 mmol) and 4,4'-azobis(4-cyanovaleric acid) (AICV; 0.4 g, 1.43 mmol) in water (10 mL) was degassed for 10 min *in vacuo* prior to being saturated with N$_2$ (bubbling with N$_2$ for 1 h). A reaction flask containing the mixture was put into a photochemical reactor (Rayonet®, and was irradiated at 254 nm for 10 h. The concentration of the irradiated mixture afforded a pale

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yellow oil which was purified with flash silica gel chromatography (10% MeOH/CH$_2$CL$_2$ to

5% HCOOH/30% MeOH/CH$_2$CL$_2$). The adduct was obtained as an oil from fractions with

Rf = 0.6 (5% HCOOH/30% MeOH/CH$_2$Cl$_2$). The combined oily material was dissolved in

MeOH (5 mL). This solution was poured slowly into ether (50 mL), that instantly led to

5   precipitation of the product as a white solid (0.5-0.6 g, 62-74%)(. ^{1}H-NMR (500.14 MHz,

CD$_3$OD): δ (ppm) 3.84-3.76 (m, 3H), 3.78 (s, 3H; COOCH$_3$), 3.70-3.48 (m, 4H), 2.60-255 (dd,

1H, J = 4.62, 13.16 Hz; H$_{3\beta}$), 2.53-2.47 (m, 4H; CH$_2$SCH$_2$) 2.36-2.35 (t, 2H, J = 7.24 Hz;

CH$_2$COOH), 2.04 (s, 3H; CH$_3$CONH), 1.87-1.80 (m, 4H; CH$_2$CH$_2$SCH$_2$CH$_2$), 1.73-1.70 (m,

1H), 1.60-1.55 (t, 1H, J = 11.89 Hz; H$_3\alpha$), 1.51-1.41 (m, 1H); FAB-MS (glycerol; negative ion

10   mode): m/z 466 [M-H]$^-$; HRMS; calcd for C$_{19}$H$_{32}$N$_1$O$_{10}$S$_1$ 466.1745, found 466.1747.
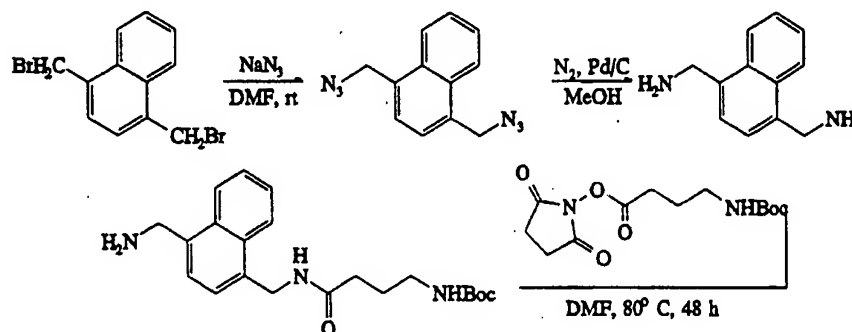
25

1,4-Bis(bromomethyl)naphthalene (Harvey, R.G. et al. 1991. *J. Org. Chem.*

56:1210) was converted to 1,4-bis(azidomethyl)naphthalene by treating with NaN$_3$ in DMF

(Blumenstein, J.J. et al. 1991. *Tetrahedron Lett.* 32:183). The azido groups of 1,4-

bis(azidomethyl)naphthalene were reduced to amino groups by catalytic hydrogenation (H$_2$

30   (1 atm), 10% Pd/C in MeOH).

1,4-Bis(azidomethyl)naphthalene: ^{1}H-NMR (400 MHz, CDCl$_3$): δ (ppm) 8.06

(m, 2H), 7.63 (m, 2H), 7.45 (s, 2H), 4.77 (s, 4H); ^{13}C-NMR (100 MHz, CDCl$_3$): δ (ppm)

132.32, 131.73, 126.97, 126.51, 124.33, 52.94; CI-MS (NH$_3$): m/z 273 (M+NH$_3$)$^-$.

1,4-Bis(aminomethyl)naphthalene: ^{1}H-NMR (300 MHz, CDCl$_3$): δ (ppm) 8.10

35   (m, 2H), 7.54 (m, 2H), 7.43 (s, 2H), 4.31 (s, 4H); ^{13}C-NMR (100 MHz, CDCl$_3$): δ (ppm)

138.31, 131.60, 126.06, 124.31, 124.08, 44.13; CI-MS (NH$_3$): m/z 187 (M+H)$^-$.

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A solution of DMF (150 mL) containing 1.4-bis(aminomethyl)

naphthalene (1.4 g, 7.53 mmol) and 4-NHBoc-butyric acid N-hydroxy-

succinimide ester (2.17 g, 7.53 mmol) was stirred at 80°C for 2 d under a stream of N_2.

After evaporation of DMF, the obtained residue was chromatographed with a silica gel (200

5   g) column by eluting with 10% MeOH/CH_2Cl_2 and then with 5% i-PrNH2/10%

MeOH/CH_2CL_2. A mono-adduct, 1-[(4'-NHBoc-1'-oxo-1'-aminobutyl)methyl]-4-

(aminomethyl)naphthalene (Rf = 0.58 in 5% i-PrNH$_2$/10% MeOH/CH_2Cl_2) was obtained in

35% yield (0.98 g). The reaction also yielded a bis-adduct, 1,4-bis[(4'-NHBoc-1'-oxo-1'-

aminobutyl) methyl] naphthalene (1.47 g). ^{1}H-NMR (300.14 MHz, CD_3OD/$CDCl_3$): δ (ppm)

10   7.98-7.95 (m, 2H), 7.51-7.49 (m, 2H), 7.33-7.32 (s, 2H), 4.81-4.77 (two s, 4H), 3.01-2.98 (t,

2H, J = 6.84 Hz), 2.18-2.14 (t, 2H, J = 6.84 Hz), 1.74-1.70 (q, 2H, J = 6.84 Hz), 1.34 (s, 9H);

FAB-MS: m/z 393 (M+Na)$^+$.
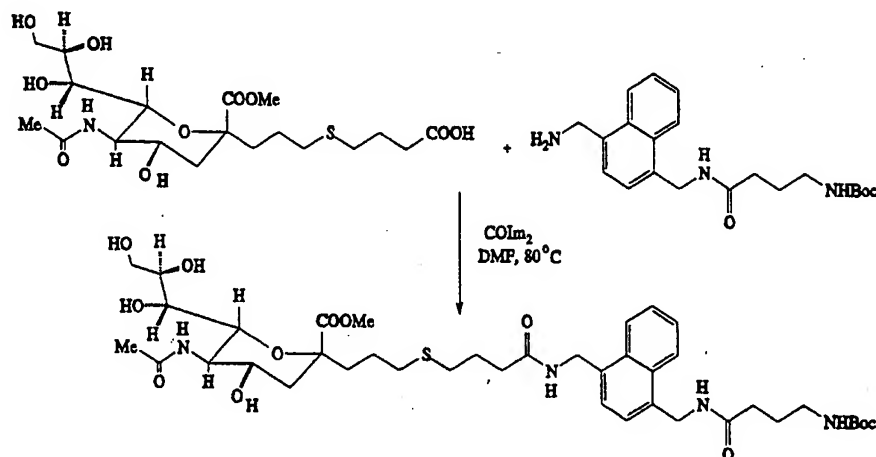
A solution of DMF (3 mL) containing a derivative of sialic acid (292 mg,

35   0.625 mmol) and carbonyldiimidazole (105 mg, 0.654 mmol) was stirred for 2 h at rt, prior to

the addition of 1-[(4'-NHBoc-1'-oxo-1'-aminobutyl)methyl]-4-(aminomethyl)naphthalene

(300 mg, 0.810 mmol). The final mixture was stirred for 2 d at 80°C under a stream of N_2.

The evaporation of DMF afforded a pale yellow residue which was then dissolved in MeOH

(10 mL) containing 5 g of ion exchange resins (Dowex-50W; H$^-$ form). After stirring of the

suspension for 5 min, a supernatant solution was separated from resins by filtration through a filter paper. The concentrate of the filtrate was purified by flash column chromatography (silica gel; 5% MeOH/CH$_2$Cl$_2$ to 20% MeOH/CH$_2$Cl$_2$). The coupling product (218 mg, 42%) was obtained as a light yellow oil (Rf = 0.71 in 20% MeOH/CH$_2$Cl$_2$). 1H-NMR (500.14

MHz, CD$_3$OD): δ (ppm) 8.07 (m, 2H), 7.55-7.53 (m, 2H), 7.41-7.39 (m, 2H), 4.90-4.85 (s, 2H), 4.82 (s, 2H), 3.95-3.71 (m, 3H), 3.70-3.47 (m, 4H), 3.73 (s, 3H; COOCH$_3$), 3.05-3.03 (t, 2H, J = 6.82 Hz), CH$_2$NHBoc), 2.55-2.45 (m, 7H), 2.25-2.23 (t, 2H, J = 6.82 Hz; ArCH$_2$NHCOCH$_2$), 2.02 (s, 3H; CH$_3$CONH), 1.85-1.65 (m, 7H), 1.62-1.58 (t, H$_{3\alpha}$, J = 12.84 Hz), 1.40 (m, 10H); FAB-MS (glycerol): m/z 721 (M+H-Boc)$^+$.
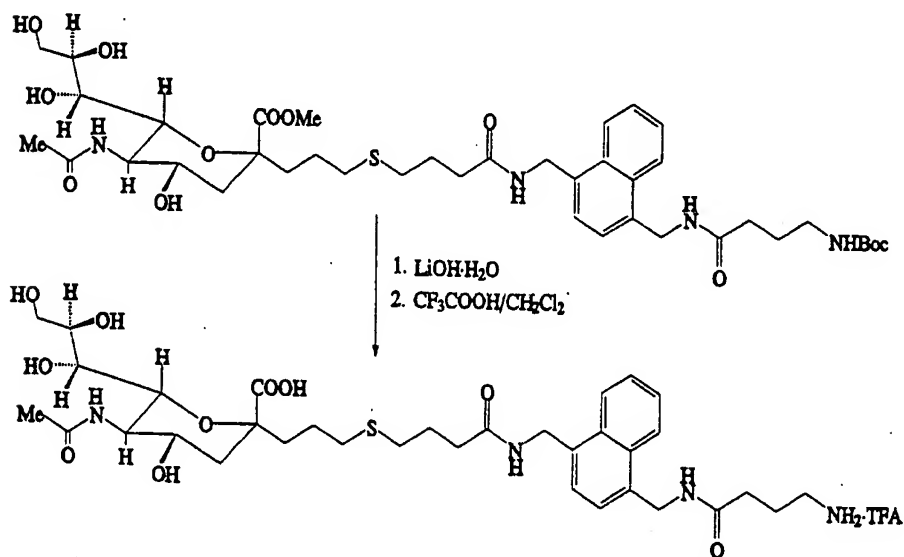
To a solution of methanol (5 mL) containing a derivative of sialic acid methyl ester (120 mg, 0.146 mmol) was added LiOH•H$_2$O (20 mg, 0.477 mmol) in H$_2$O (5 mL). After stirring (24 h at rt), the reaction mixture was adjusted to be acidic (pH ~ 3) by adding ion exchange resins (Dowex-50W; H$^+$ form). After filtration of resins, the filtrate was concentrated *in vacuo* to afford an oily residue: this material was pure enough to proceed to next step without further purification. ^{1}H-NMR (500.14 MHz, CD$_3$OD): δ (ppm) 8.06 (m, 2H), 7.57-7.54 (m, 2H), 7.42-7.41 (m, 2H), 4.85-4.81 (two s, 4H), 3.85-3.70 (m, 3H), 3.68-3.50 (m, 4H), 3.05-3.02 (t, 2H, J = 6.7 Hz; CH$_2$NHBoc), 2.62-2.59 (m, H3β), 2.55-2.48 (m, 4H; CH$_2$SCH$_2$), 2.40-2.38 (t, 2H, J = 7.23 Hz; CH$_2$CONH), 2.26-2.22 (t, 2H, J = 7.16 Hz; NHCOCH$_2$), 2.04 (s, 3H); CH$_3$CONH), 1.86-1.82 (m, 5H), 1.76-1.74 (t, 2H, J = 7.16 Hz),

1.59-=1.58 (t, H3α, J = 11.0 Hz), 1.40 (m, 10H); FAB-MS (glycerol; negative ion mode): m/z 805 [M-H]; HRMS: calcd for $C_{39}H_{57}N_4O_{12}S_1$ 805.3690, found 805.3964; calcd for $C_{39}H_{58}N_4O_{12}S_1Na_1$ 829.3666, found 829.3670. To an N-Boc protected, derivative of sialic acid (50 mg, 0.062 mmol)(suspended in CH_2Cl_2 (1 mL) at 0°C was added a mixture of

5   CF_3CO_2H (1mL) and CH_2Cl_2 (1 mL). The mixture was stirred for 1 h at 0°C. The mixture (a pale red solution) was poured slowly into cold ether (50 mL), which led to formation of a white precipitate. It was collected by centrifugation and washed with ether (50 mL). The yield of deprotection of N-Boc was estimated to be quantitative on the basis of ^{1}H-NMR (CD_3OD) spectrum of the product (NeuAc-L_4-NH_2(4) as TFA salt). FAB-MS (glycerol;

10   negative ion mode): m/z 705 [M-H].

EXAMPLE 2.

This example illustrates the application of polymeric polyvalent galactosides

15   to the inhibition of the adhesion of ricins to erythrocytes. This demonstration is significant in establishing the activity of polyvalent inhibitors against a molecular toxin (rather than viral or cellular) target.

Two derivatives of D-galactosides (Gal-$β_O$-L_1NH_2, Gal-$α_C$-L_2NH_2) were synthesized as monomeric precursors to polymeric polyvalent D-galactosides: Gal-$β_O$-L_1NH_2

20   contains a β-O-linkage between the galactoside (Gal) group and the amine-terminated linker (see Figure 4); Gal-$α_C$-L_2NH_2 contains an α-C-glycoside. As a C_1-epimeric analog of β-O-galactosides, the Gal-$α_C$-L_2NH_2 was chosen because it could be prepared easily in high stereoselectivity and large scale. The C-glycosidic linkage of Gal-$α_C$-L_2NH_2 provides the additional advantage of resistance to chemical and enzymatic hydrolysis. Both epimers of D-

25   galactoside were used to compare their binding affinities to ricins. Two types of polymers-poly(acrylic acid) (pAA; M_w = ~ 140 kDa, M_w/M_n = 1.91) (Mammen, et $al.$, $J.$ $Med.$ $Chem.$, 1995, 38, 4179-4190) and poly(butadiene-co-maleic acid) (pBMA; M_w = 10-15 kDa) were used as polymeric scaffolds to present multiple copies of monovalent galactosides as amide side chains; these polymers are referred to as pAA(Gal-β), pAA(Gal-α),pBMA(Gal-β), and

30   pBMA(Gal-α). The pAA-based polymers were expected to be relatively flexible, at least at

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high ionic strength; the pBMA-derived polymers were expected to be less flexible.

pAA(Gal-β) was synthesized using methods described previously by allowing poly(*N*-

acryloyloxysuccinimide) (pNAS) (Mammen, *et al., J. Med. Chem.,* 1995, *38,* 4179-4190) to

react with Gal-β$_0$- L$_1$NH$_2$ in DMF (~ 20°C, 2 d), and quenching with excess 1.0 M NaOH. By

5   allowing pNAS to react with χ equivalents of Gal-ß$_0$-L$_1$NH$_2$ per equivalent of active ester

groups on the polymer (χ = 0.2, 0.4, 0.6, 0.8, and 1.0), various pAA(Gal-β; 0.2 to 1.0) were

prepared. The parameter χ is also equivalent to the mole fraction of Gal in the polymers, and

is defined as the number of side chains containing Gal divided by the total number of side

chains (see Figure 3). Applying the same strategy to poly(butadiene-*co*-maleic anhydride)

10   (pBMAn) generated pBMA (Gal-β; 0.05 to 0.22) and pBMA(Gal-α; 0.05 to 0.22). All the

polymers were purified by dialysis (MW cutoff ~ 3.5 kDa), and characterized by means of

^{1}H-NMR spectroscopy and combustion analysis (sulfur). The yields of amide-forming

reactions were ≥95% and ≥65% for pNAS and pBMAn, respectively, on the basis of the

combustion analysis of the polymers.

15   **Inhibition of Adhesion of Ricins to Erythrocytes by Galactoside-**

Presenting Polymers. Red blood cells from 2-week-old chick were used as a model system

of mammalian cells. Erythrocytes lack a nucleus, and do not synthesize proteins.

Nevertheless, they provide a good model of the cells targeted by ricin, and provide a system

with which to study the adhesion of ricin to the cell surface: the surface of erythrocytes

20   presents a variety of ß-galactoside-containing glycoconjugates (~ 2-3 x 10^6 Gal residues per

human RBC). Ricins attach to chick RBCs, and cause their aggregation and lysis. Labeling

the ricins with fluorescent isothiocyanate (FITC) established that aggregation and lysis were

due to the action of the ricins. A polyvalent galactoside, pAA(Gal-β; 0.4) prevents these

effects.

25   Using chick erythrocytes (0.5% by volume) as a suspension in phosphate

buffered saline (PBS) solution, pH 7.2 and ricins (RCA$_{120}$ ~ 16 nM; RCA$_{60}$ ~ 1.9 μM), the

activities of polymeric galactosides in inhibiting ricin-mediated agglutination was assayed.

Table IV (Appendix) summarizes the hemagglutination inhibition (HAI) activity, K$_i^{HAI}$

(defined as the lowest concentration of an inhibitor required to prevent hemagglutination) of

30   purified polymers with various mole fractions of carbohydrate-containing side chains

($\chi^{carbohydrate}$, defined in Figure 4). Monovalent Gal-β_O-L_1NH_2 gave values of K_i^{HAI} 5- and 3-fold lower than those of Gal-β-OMe against RCA_{120} and RCA_{60}, respectively. Monovalent galactosides containing a β-O-anomeric configuration gave activities better than the corresponding α-galactosides, though the difference was not great. This observation implies that the binding of Gal residues to ricin Gal-binding sites is not highly sensitive to the anomeric configuration ($\beta \geq \alpha$) or to the nature of atom ($O \geq C$) attached to the anomeric carbon.

PAA(Gal-β; 0.2 to 1.0) showed HAI activity, K_i^{HAI}, against RCA_{120}-induced agglutination at submicromolar concentrations of Gal moieties in solution.(Appendix, Table IV). The same polymers had HAI activities against RCA_{60} that were ~ 50- to ~ 300-fold lower than those against RCA_{120}; the activities against RCA_{60} were, at best, 50 times better than those of monomeric Gal derivatives. By contrast, pAA(Gal-β; 0.4) had an inhibitory activity (against RCA_{120}) that was ~ 1500 times higher than that of monovalent Gal-β-OMe, and ~ 270 times higher than that of Gal-β_O-L_1NH_2. pBMA(Gal-β; 0.05 to 0.22) showed HAI activities at (sub)micromolar concentrations against RCA_{120} and RCA_{60}; the activities against RCA_{120} were better than those against RCA_{60}. pBMA(Gal-α; 0.05 to 0.22) had, however, HAI activities against RCA_{60} approximately 4 times better than those against RCA_{120}. Of the polyvalent galactosides built on pBMA, pBMA(Gal-β; 0.22) and pBMA(Gal-β; 0.05) were the most active inhibitors against RCA_{120} and RCA_{60}, respectively. pBMA(Gal)s are relatively small polymers with molecular weight ~ 10-15 kDa, and have a polymer backbone (pBMA) that is biocompatable (Conroy, *et al., Bioorg. Chem.*, 1996, *24*, 262-272).

Other polymers were tested presenting multiple copies of non-galactoside carbohydrates as controls (pAA(NeuAc-α; 0.2) (Mammen, *et al., J. Med. Chem.*, 1995, *38*, 4179-4190), pBMA (NeuAc-α; 0.2 to 1.0), pAA(GlcNAc-β; 0.2 to 1.0)). None of them inhibited the agglutination of erythrocytes by ricin at concentrations comparable to those in Table IV ($K_i^{HAI} > 300\mu M$). It was concluded that ricin-induced agglutination of erythrocytes was inhibited selectively by polymers presenting galactosides. Both α and β anomers seem to be approximately equal in effectiveness. These results suggest that the inhibition by polymeric galactosides is primarily due to the specific binding of galactoside ligands to the Gal receptor sites of ricins. We believe that the high activity of polymeric polyvalent

galactosides relative to monovalent galactosides is partly due to multivalent (entropically-enhanced) binding of galactoside ligands to multiple receptor sites.

The HAI activity of the polymers against ricins is summarized in Figures 4a and 4b. Figure 4a (with RCA_{120}) shows that values of K_i^{HAI} of polymeric galactosides are a

5 non-linear function of the mole fraction of Gal (χ^{Gal}) of the polymers. The relationship between activity and χ^{Gal} of the polymers in inhibiting hemagglutination by RCA_{60} seem to be related closely to that describing inhibition by RCA_{120}. The K_i^{HAI}-χ^{Gal} relationship of the polymers depended also on the type of polymer backbone: a smooth curve with large, flexible pAA(Gal-β); a sharp, partly quasi-linear relationship with small, extended

10 pBMA(Gal). pAA(Gal-β; 0.01) (degree of polymerization = DP ~ 2000), presenting a low density of Gal side chains (~ 20 Gals per polymer chain), still showed an activity (K_i^{HAI}~ 2.0 μM), that was greater than that of monovalent Gal-β-L_1NH_2 by a factor of 19. The increased activity of pAA(Gal-β; 0.01) relative to monomeric Gal-β-L_1NH_2 is ascribed to the flexibility of its polymer backbone, and thus to its ability to adjust to the distances between galactoside

15 ligands; this flexibility should facilitate multivalent binding to the receptor sites of ricin.

Under the conditions used in assaying the Gal-presenting polymers for their ability to inhibit agglutination, the titer of RCA_{120} - a minimal concentration that could agglutinate RBCs (200 μL of 0.25% by volume, suspended in PBS solution) was 4 nM, and that of RCA_{60} was 480 nM. This difference indicates that RCA_{60} (one B-chain; ~ 3 Gal

20 receptor sites) is more weakly agglutinating than is RCA_{120} (two B-chains; ~ 6 Gal receptor sites) (Lord, et al., FASEB, 1994, 8, 201-208; Frankel, et al., Biochem., 1996, 35, 14749-14756). Monovalent galactosides were more effective against RCA_{60} than against RCA_{120} in preventing the ricin-induced agglutination of erythrocytes. Since the inhibition of agglutination reflects the competitive binding of monovalent galactosides to the Gal receptor

25 sites of ricin, this difference in activities suggested at least two hypotheses regarding adhesion of ricins to cells. Not being bound to theory, the difference in the observed HAI activities of monomeric galactosides against RCA_{60} and against RCA_{120} can be due to differences in the intrinsic affinities of monomeric galactosides for Gal receptor sites on RCA_{60} and RCA_{120}. Binding studies of several monomeric derivatives of galactosides with

30 the two ricins using equilibrium dialysis and fluorescence techniques, however, showed that

the two ricins had similar affinities for the monovalent galactosides (within a factor less of than 10). Otherwise, the low values of K_i^{HAI} of monovalent galactosides against RCA_{60} relative to RCA_{120} can be related to the number (n) of blocked Gal receptor sites of ricins required for inhibition of agglutination relative to the total number of Gal receptor sites of ricins: n/3 (RCA_{60}); n/6 (RCA_{120}). That is, blocking of the same number of Gal sites on RCA_{60} and RCA_{120} results in a larger fraction of Gal sites on RCA_{60} being blocked, and is more effective in abolishing the agglutination ability of RCA_{60} than of RCA_{120}.

Polymeric galactosides gave values of K_i^{HAI} that were lower for RCA_{120} than for RCA_{60}. The relative enhancement of the activities of galactoside moieties on polyvalent presentation (that is, the value of K_i^{HAI} for the polymer relative to that of the monomer) was higher for RCA_{120} than for RCA_{60}, as well. These results indicate that polyvalent ligands are more effective as inhibitors against a target with a higher valency (RCA_{120}) than against one with a lower valency (RCA_{60}).

With RCA_{60}, some of the polymers (pAA(Gal-β; 0.6 to 1.0), pBMA(Gal-β; 0.17, 0.22), pBMA(Gal-α; 0.05)) were *less* effective in blocking ricin-mediated agglutination (on a per Gal basis) than were the corresponding monovalent galactosides. Similar effects have been observed in two other systems: (i) Sialoside ligands presented as side chains on poly(acrylamide) bound less tightly (on a per-sialoside basis) to bromelain-cleaved hemagglutinin (BHA) than did the same monomeric ligands not attached to the polymer; BHA is a homotrimer with three independent sialoside-binding sites. (ii) Oxamate ligands presented on poly(acrylamide) were less effective in preventing the agglutination of RBCs by lactic dehydrogenase (LDH) than were monomeric oxamate ligands not attached to the polymer; LDH is a homotetrameric complex with four independent oxamate-binding sites. These results indicate that for some polymeric polyvalent inhibitors there may not be significant entropic enhancement of binding to the small number of receptor sites presented by BHA (3 receptor sites), LDH (4 receptor sites) and RCA_{60} (~ 3 receptor sites) and that ligands presented on polymer backbone may have lower affinities for a receptor than do these ligands without the encumbering polymer (possibly due to undefined interactions between the polymer backbone and the protein).

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The fact that many of the polymers were *more* effective in blocking ricin-mediated agglutination (on a per Gal basis) than were the corresponding monovalent galactosides suggests that factors other than the fractional occupancy of receptor sites determine K_i^{HAI}. Since each polymer presents multiple Gal moieties, the binding of polymer to ricin may, of course, be relatively tight, even if individual Gal groups bind relatively weakly. The experiments presented here do not directly measure the occupancy of the Gal receptor sites on ricin, and the inhibition of hemagglutination may therefore be due either to entropically-enhanced binding of these Gal-binding receptor sites, or to other, non-receptor directed effects such as steric inhibition (Choi, *et al., Chem. Biol.*, 1996, *3*, 97-104; Mammen, *et al., J. Med. Chem.*, 1995, *38*, 4179-4190).

This example demonstrates that synthetic polymers presenting multiple copies of a simple derivative of β-D-galactose as side chains can effectively inhibit adhesion of ricins to chicken erythrocytes.

General Procedure. All common chemicals were used as received from suppliers without further purification, unless otherwise noted. Red blood cells (RBCs or erythrocytes) from 2-week-old chicks were purchased from Spafas Inc. The erythrocytes, which were provided as a suspension (~ 5% v/v) in a storage buffer, were washed 4 times with phosphate buffered saline (PBS; 137 mM NaCl, 2.68 mM KCl, 7.75 mM Na_2HPO_4, 1.47 mM KH_2PO_4), pH 7.2 and then re-suspended in PBS (~ 0.5% v/v) as described (Choi, *et al., Chem. Biol.*, 1996, *3*, 97-104; Mammen, *et al., J. Med. Chem.*, 1995, *38*, 4179-4190). Ricins (RCA_{120}, RCA_{60}) including fluorescein isothiocyanate (FITC)-labeled ricins (FITC-labeled RCA_{120}, FITC-labeled RCA_{60}) were purchased from Sigma Co.

Synthesis of Gal-β₀-L₁NH₂ and Gal-α_C-L₂NH₂ (see Figure 3). Gal-β₀-L₁NH₂: To a solution of methylene chloride (180 mL) containing β-D-galactose pentaacetate (7.8 g, 19.98 mmol) and allyl alcohol (5.6 ml, 58.82 mmol) cooled in ice bath $BF_3.Et_2O$ (4.0 mL, 32.52 mmol) was added dropwise (Liu, *et al., Carbohydr. Res.*, 1996, *290*, 233-237). After stirring (4 h, 0°C; then 30 h, ~ 20°C), the mixture was poured into cold saturated $NaHCO_3$ (200 mL) in a separatory funnel. After shaking, the organic layer was separated out, and washed again with cold saturated $NaHCO_3$ (200 mL). After drying over $MgSO_4$, the methylene chloride solution was evaporated *in vacuo* to yield a pale yellow oil (R_f = 0.57 in

5% MeOH/CH_2Cl_2). The crude products were dissolved in methanol (100 mL), followed by addition of LiOH (2.88 g, 120 mmol) in water (50 mL). After stirring (12 h, ~ 20°C), the reaction mixture was neutralized by adding 6.0 M HCl (~ 20 mL). The aqueous mixture was concentrated *in vacuo* to afford a thick oily residue, which was purified with flash column chromatography (silica gel; 5% to 40% MeOH/CH_2Cl_2). The product, Gal-β_O-$CH_2CH=CH_2$, was obtained in 91% (4.0 g) yield over two steps as a pale yellow oil (R_f = 0.80 in 30% MeOH/CH_2Cl_2). ^{1}H-NMR (300.1 MHz, CD_3OD): δ (ppm) 6.00-5.89 (m, 1H), 5.36-5.29 (dd, J = 17.2, 3.2, 1H), 5.18-5.12 (dd, J = 12.1, 3.2 Hz, 1H), 4.40-4.34 (dd, J = 13.0, 5.2 Hz, 1H), 4.27-4.25 (d, J = 7.3 Hz, 1H; H_{1ax}), 4.17-4.10 (dd, J = 13.0, 6.1 Hz, 1H), 3.90-3.83 (m, 1H), 3.80-3.68 (m, 3H), 3.56-3.50 (m, 2H); FAB-MS (glycerol): m/z 221 [M+H]$^+$; HRMS: calcd for $C_9H_{17}O_6$ 221.1024, found 221.1025. A solution of Gal-β_O-$CH_2CH=CH_2$ (4.0 g, 18.17 mmol), $HSCH_2CH_2NH_2$.HCl (6.19 g, 54.5 mmol), and 4,4'-azobis(4-cyanovaleric acid) (0.4 g, 1.43 mmol) in water (50 mL)-methanol (5 mL) was degassed for 10 min *in vacuo* prior to being saturated with N_2 (by bubbling N_2 gas through the solution for 30 min). A reaction flask containing the mixture was placed in a photochemical reactor (Rayonet®), and was irradiated at 254 nm for 10 h. The irradiated mixture was neutralized by adding 2.0 M NaOH (28 mL), and immediately evaporated to remove volatiles. The evaporation yielded a pale yellow oil, which was purified with flash silica gel chromatography (10% MeOH/CH_2Cl_2 to 5% *i*-PrNH$_2$/40% MeOH/CH_2Cl_2). The adduct (Gal-β_O-L$_1$NH$_2$) was obtained as an oil (R_f = 0.33 in 5% *i*-PrNH$_2$/30% MeOH/CH_2Cl_2). ^{1}H-NMR (250.1 MHz, CD_3OD): δ (ppm) 4.22-4.19 (d, J = 7.4 Hz, 1H; H_{1ax}), 4.0-3.95 (ddd, J = 9.9, 6.1, 6.1 Hz, 1H), 3.87-3.81 (m, 1H), 3.78-3.63 (m, 4H), 3.53-3.43 (m, 2H), 2.81-2.76 (t, J = 6.5 Hz, 1H), 2.67-2.59 (q, J = 7.1 Hz, 4H), 1.92-1.82 (quin, J = 6.6 Hz, 2H); ^{13}C-NMR (100.6 MHz, CD_3OD): δ (ppm) 76.6, 75.8, 72.5, 70.3, 69.2, 62.5, 41.6, 35.6, 31.2, 29.0; FAB-MS (glycerol): m/z 298 [M+H]$^+$; HRMS: calcd for $C_{11}H_{24}NO_6S$ 298.1323, found 298.1324.

Gal-α_C-L$_2$NH$_2$: Gal-α_C-$CH_2CH=CH_2$ was prepared by α-*C*-allylation of β-D-galactose pentaacetate (Giannis, *et al.*, *Tetrahedron Lett.*, 1985, *26*, 1479-1482), and subsequent hydrolysis of the acetates. ^{1}H-NMR (400.0 MHz, CD_3OD): δ (ppm) 5.91-5.83 (m, 1H), 5.13-4.99 (m, 2H), 4.00-3.96 (m, 2H), 3.95-3.88 (m, 1H), 3.76-3.67 (m, 4H), 2.49-2.34 (m, 2H); ^{13}C-NMR (100.6 MHz, CD_3OD): δ (ppm) 136.7, 116.9, 75.7, 74.0, 71.9, 70.1, 70.0,

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62.0, 31.0; FAB-MS (glycerol): m/z 227 [M+Na]$^+$. Gal-α_C-L$_2$NH$_2$ was prepared from Gal-α_C-

CH$_2$CH=CH$_2$ as described above. ^{1}H-NMR (400.0 MHz, CD$_3$OD): δ (ppm) 3.95-3.63 (m,

7H), 3.12 (t, J = 6.9, 2H), 2.81 (t, J = 3.4 Hz, 2H), 2.66-2.59 (m, 2H), 1.81-1.62 (m, 4H); ^{13}C-

NMR (100.6 MHz, CD$_3$OD): δ (ppm) 75.7, 74.0, 71.9, 70.4, 70.2, 62.5, 39.9, 32.1, 26.8, 24.8,

5   20.9; FAB-MS (glycerol): m/z 282 [M+H]$^+$; HRMS: calcd for C$_{11}$H$_{24}$NO$_5$S 282.1375, found

282.1374.

      Synthesis of pAA(Gal-β), pBMA(Gal-β), and pBMA(Gal-α) (Figure 3).

pAA(Gal-β): A method for synthesis of pAA(Gal-β; 0.4) is described here as a protocol for

general synthesis of pAA(Gal). To a solution of N,N-dimethylformamide (DMF, 8 mL)

10   containing poly(N-acryloyloxysuccinimide) or pNAS (500 mg, equivalent to 3 mmol of

NAS) (Mammen, *et al., J. Med. Chem.,* 1995, *38,* 4179-4190) was added Gal-β$_O$-L$_1$NH$_2$ (356

mg, 1.2 mmol) dissolved in DMF (2 mL), followed by addition of i-Pr$_2$NEt (0.2 mL, 1.2

mmol). After stirring (2 d, ~ 20 °C), the mixture was basified by adding 1.0 M NaOH (3

mL), followed by stirring for additional 2 h at ~ 20°C. At the conclusion of reaction, the

15   mixture was transferred into a dialysis bag (MW cutoff ~ 12-14 kDa; Spectrum®), and was

dialyzed at ~ 20°C over 3 d: H$_2$O (2 x 4L), 0.05 M NaOH (4L), 0.5 M NH$_4$Cl (4L), and H$_2$O

(2 x 4L). The content of the bag was lyophilized to afford pAA(Gal-β; 0.4) which was

obtained as a fluffy white solid (499 mg). ^{1}H-NMR (500.1 MHz, D$_2$O): δ (ppm) 4.3 (d, J =

7.5 Hz; H$_{1ax}$), 3.89 (br s), 3.85 (s), 3.8-3.5 (m), 3.4 (br m), 3.3 (br s), 2.6 (br s), 2.2-1.9 (br d),

20   1.7-1.3 (br m); % S: calcd for pAA(Gal-β; 0.4) 6.97, found 6.71. Other pAA(Gal-β)s were

prepared following the same procedures. % S: calcd for pAA(Gal-β; 0.2) 5.00, found 5.12;

calcd for pAA(Gal-β; 0.6) 8.02, found 8.06; calcd for pAA(Gal-β; 0.8) 8.67, found 8.58; calcd

for pAA(Gal-β; 1.0) 9.11, found 9.05.

      pBMA(Gal-β) and pBMA(Gal-α). These polymers were synthesized following

25   the above protocol with a slight difference, in which poly(butadiene-*co*-maleic anhydride) or

pBMAn was used instead of pNAS as a precursor polymer. An aliquot of pBMAn, which

was provided as a solution in acetone (Polysciences, Inc.), was dried *in vacuo* and

redissolved in DMF before use. pBMA(Gal-β; 0.09): ^{1}H-NMR (500.1 MHz, D$_2$O): δ (ppm) 5.6

(br s), 5.4 (br s), 3.9-3.5 (br m), 3.4-3.3 (br m), 3.0-2.9 (br m), 2.7-2.5 (br m), 2.4-2.1 (br s),

30   1.8 (br s), 3.9-3.5 (br m). pBMA(Gal-α; 0.09): ^{1}H-NMR (500.1 MHz, D$_2$O): δ (ppm) 5.6 (br s),

5.5-5.3 (br d), 4.3-3.7 (br m), 3.6 (br m), 3.3 (br s), 2.9 (br s), 2.8-2.4 (br s), 2.4-2.0 (br s),

1.8-1.4 (br m), 1.0 (br s). % S: calcd for pAA(Gal-α; 0.05) 1.62, found 1.55; calcd for

pAA(Gal-α; 0.09) 2.62, found 2.69; calcd for pAA(Gal-α; 0.17) 4.11, found 4.12; calcd for

pAA(Gal-α; 0.22) 4.81, found 4.85.

5   **Ricin-mediated Agglutination of Chick RBCs, and Its Prevention by**

pAA(Gal). (i) Adhesion of ricin to chick RBCs: A suspension of RBCs (0.5% v/v; 0.4 mL)

in PBS, pH 7.2 was mixed well with PBS solution of fluorescent ricin (0.4 mL of FITC-

labeled RCA_{120} (40 nM) or FITC-labeled RCA_{60} (1.4 μM)) contained in an 1-mL Eppendorf

vial. After incubation for 2 h at 4°C, the mixture was centrifuged for 2 min at 2000 rpm.

10   After discarding a supernatant solution, red pellets were washed with 1.0 mL of PBS, and

resuspended gently in PBS (0.2 mL). Optical images of absorption and fluorescence of

RCA-adsorbed RBCs were obtained by taking an aliquot of suspended pellets on a glass

slide, and examining the sample with optical and fluorescence microscopy (Leica DMRX).

(ii) Protection of RBCs by pAA(Gal-β) from the cellular adsorption of ricin: A solution of

15   FITC-labeled RCA_{120} (80 nM; 0.2 mL) in PBS was mixed with a PBS solution (0.2 mL) of

pAA(Gal-β; 0.4) (90 μg mL^{-1} or [Gal] = 200 μM) in an Eppendorf vial. After incubation (30

min, 4°C), the ricin-polymer mixture was added to a suspension of RBCs (0.5% v/v; 0.4 mL)

in PBS, followed by gentle agitation and incubation for 2 h at 4°C. The incubated mixture

was centrifuged for 2 min at 2000 rpm. Red pellets, obtained after removing the supernatant,

20   were washed with 1.0 mL of PBS, and resuspended in 0.2 mL of PBS before being examined

with an optical microscope.

Hemagglutination (ricin-induced) Inhibition (HAI) Assay. The titer of the

prepared PBS solution of ricins (RCA_{120} = 16 nM; RCA_{60} = 1.9 μM) was determined by 2-

fold serial dilution of 50 μL of the ricin solution (mg mL^{-1}) through 12 wells (8 x12-well

25   microtiter plate with conically-shaped bottoms; ICN Flow). Another 50 μL PBS was added

to each well, followed by addition of a suspension of chicken erythrocytes in PBS (100 μL).

The solution was mixed and incubated at ~ 20 °C for 1 h. The end point of hemagglutination

(HA) is defined as the last well in which a sufficient amount of ricin remains to agglutinate

the erythrocytes. A stock PBS solution (50 μL) of polymeric galactoside (1-2 mg mL^{-1}; [Gal]

30   ~ 2-6 mM) or monovalent galactoside (5 mM) was 2-fold serially diluted through 12

microtiter wells containing 50 μL of PBS. After serial dilutions of solutions of polymeric or monomeric galactoside, each well (50 μL) was mixed with 50 μL of RCA$_{120}$ (16 nM) or RCA$_{60}$ (1.9 μM). After 30 min of incubation at ~ 20°C, 100 μL of a suspension of chicken erythrocytes (0.5% v/v) was added to each well followed by gentle agitation and incubation

5   (1 h, ~ 20°C). The end point of HAI is the last well in which an agglutinated pellet is observed. This end point (K_i^{HAI}) is defined as the lowest concentration of galactoside in solution that inhibited the ricin-induced agglutination of erythrocytes. The values of K_i^{HAI} were calculated on the basis of at least 5 independent trials.

10   **EXAMPLE 3**: This example illustrates the generation of pMVMA(NeuAc) using quasi-solid phase reaction (*see* Figure 5a).

          Solutions of co-polymers of poly(methyl vinyl ether-*co*-maleic acid)(NeuAc-L$_1$), or pMVMA(NeuAc-L$_1$) were prepared by reacting of RNH$_2$ (NeuAc-L$_1$NH$_2$) with poly(methyl vinyl ether-*co*-maleic anhydride), or pMVMAn using

15   different molar equivalent (mol equiv) of RNH$_2$ to anhydride groups of pMVMAn (mol equiv = {number of moles of RNH$_2$}/{number of moles of anhydride groups of pMVMAn}) and using aqueous solutions of amines adjusted to pH 12. Co-polymeric pMVMA(NeuAc) for which mol equiv is >0 was generated in microtiter plates with 96 conically-bottomed wells as follows: (i) placing 3 mg of pMVMAn (M$_n$ = 67000, 80000, 311000, 485000, or

20   1130000 gmol^{-1}) into a well; (ii) soaking the powder with a variable amount (10-38 μL) of 0.1 M of RNH$_2$ (NeuAc-L$_1$NH$_2$) in PBS buffer, pH 12; (iii) immediately sealing the plate, and then ultrasonicating the mixture for 0.5 h. Each solution of co-polymers (pH ~ 3) generated in a well was neutralized to pH ~ 7 by adding 30 mL of 1.0 M NaOH and adjusted to 100 or 200 μL (total volume) with PBS, pH 7.2, before the inhibition assay of influenza

25   virus-induced agglutination of chicken red blood cells.

          EXAMPLE 4: This example illustrates the generation of pMVMA(NeuAc;R) using quasi-solid phase reaction (*see*, Figure 5b).

          The protocol used in Example 3 was extended to the preparation of

30   ter-polymers pMVMA(NeuAc-L$_1$; R). A three-component mixture pMVMAn,

NeuAc-L_1NH_2 and R_2NH_2(aliphatic amines, aromatic amines, amino acids, aminosugars, or

peptides) was sonicated. Ter-polymeric pMVMA(NeuAc;R_2) for which mol equiv of

NeuAc-L_1NH_2 and R_2NH_2 to anhydride group of pMVMAn are >0 was generated in

microtiter plates with 96 conically-bottomed wells as follows: (i) placing 3 mg of pMVMAn

5   (M_n = 67000, 80000, 311000, 485000, or 1130000 gmol^{-1}) into a well; (ii) soaking the

powder with 10 μL of 0.1 M NeuAc-L_1NH_2, and a variable amount (2-20 μL) of 0.1 M of

RNH_2 (examples: naphthylalanine, phenylalanine, cyclohexylamine, phenylethylamine,

4-aminobenzoic acid, or mannosamine) in PBS buffer, pH 12; (iii) immediately sealing the

plate, and then ultrasonicating the mixture for 0.5 h. Each solution of ter-polymers (pH ~ 3)

10   generated in a well was neutralized to pH ~ 7 by adding 30 μL of 1.0 M NaOH and adjusted

to 100 or 200 μL (total volume) with PBS, pH 7.2, before the inhibition assay of influenza

virus-induced agglutination of chicken red blood cells.

        EXAMPLE 5: This example illustrates the generation of pAA(Gal) using

15   quasi-solid phase reaction (see, Figure 5c).

        Solutions of co-polymers of pAA(Gal) were prepared by reacting of RNH_2

(Gal-β-L_2NH_2; Gal-α-L_3NH_2) with poly(acrylic anhydride) (pAAn; M_n = 20700 gmol^{-1}, M_w =

39500 gmol^{-1}) using different molar equivalent (mol equiv) of RNH_2 to anhydride groups of

pAAn (mol equiv = {number of moles of RNH_2}/{number of moles of anhydride groups of

20   pAAn}) and using aqueous solutions of amines adjusted to pH 12. Co-polymeric pAA(Gal)

for which mol equiv is >0 was generated in microtiter plates with 96 conically-bottomed

wells as follows: (i) placing 6 mg of pAAn into a well; (ii) soaking the powder with a

variable amount (10-100 μL) of 0.1 M of RNH_2 (Gal-β-L_2NH_2, or Gal-α-L_3NH_2) in PBS buffer,

pH 12; (iii) immediately sealing the plate, and then ultrasonicating the mixture for 0.5 h.

25   Each solution of co-polymers (pH ~ 3) generated in a well was neutralized to pH ~ 7 by

adding 60 μL of 1.0M NaOH and adjusted to 100 or 200 μL (total volume) with PBS, pH

7.2, before the inhibition assay of ricin-induced agglutination of chicken red blood cells.

        EXAMPLE 6: This example illustrates the generation of pBMA(Gal) using

30   quasi-solid phase reaction (see Figure 5d).

The protocol used in Example 5 was extended similarly to the preparation of
co-polymers of poly(butadiene-*co*-maleic acid)(Gal), or pBMA(Gal). Solutions of
co-polymers of pBMA(Gal) were prepared by reacting of RNH_2 (Gal-β-L_2NH_2; Gal-α-L_3NH_2)
with poly(butadiene-*co*-maleic anhydride) (pBMAn; M_w = 10000-15000 gmol^{-1}) using

5   different molar equivalent (mol equiv) of RNH_2 to anhydride groups of pBMAn (mol equiv =
{number of moles of RNH_2}/{number of moles of anhydride groups of pBMAn}) and using
aqueous solutions of amines adjusted to pH 12. Co-polymeric pBMA(Gal) for which mol
equiv is >0 was generated in microtiter plates with 96 conically-bottomed wells as follows:
(i) placing 6 mg of pBMAn into a well; (ii) soaking the polymer with a variable amount

10   (10-100 μL) of 0.1 M of RNH_2 (Gal-β-L_2NH_2, or Gal-α-L_3NH_2) in PBS buffer, pH 12; (iii)
immediately sealing the plate, and then ultrasonicating the mixture for 0.5 h. Each reaction
mixture of co-polymers (pH ~ 3) generated in a well was neutralized to pH ~ 7 by adding 60
μL of 1.0 M NaOH and adjusted to 100 or 200 μL (total volume) with PBS, pH 7.2, before
the inhibition assay of ricin-induced agglutination of chicken red blood cells.

15

EXAMPLE 7: This example illustrates the generation of pAA(SLe^x) using
quasi-solid phase reaction (see Figure 6a).

The protocol used in Example 5 is extended similarly to the
preparation of co-polymers of pAA(SLe^x). Solutions of co-polymers of pAA(SLe^x) are

20   prepared by reacting RNH_2 (SLe^x-NH_2) with pAAn using different molar equivalent (mol
equiv) of RNH_2 to anhydride groups of pAAn (mol equiv = {number of moles of
RNH_2}/{number of moles of anhydride groups of pAAn}) and using aqueous solutions of
amines adjusted to pH 12. Co-polymeric pAA(SLe^x) for which mol equiv is >0 is generated
in microtiter plates with 96 conically-bottomed wells as follows: (i) placing 6 mg of pAAn

25   (M_n = 20700 gmol^{-1}, M_w = 39500 gmol^{-1}) into a well; (ii) soaking the polymer with a variable
amount (10-100 μL) of 0.1 M of RNH_2 (SLe^x-NH_2) in PBS buffer, pH 12; (iii) immediately
sealing the plate, and then ultrasonicating the mixture for 0.5 h. Each reaction mixture of
co-polymers (pH ~ 3) generated in a well is neutralized to pH ~ 7 by adding 60 mL of 1.0 M
NaOH and adjusted to 100 or 200 μL (total volume) with PBS, pH 7.2, before the inhibition

30   assay of adhesion of neutrophils to endothelial cells.

EXAMPLE 8: This example illustrates the generation of pAA(Bacitracin;R) using quasi-solid phase reaction (see Figure 6b).

The protocol used in Example 4 is extended to similarly to the preparation of ter-polymers pAA(Bacitracin;R). A three-component mixture including pAAn, Bacitracin and R_2NH_2(aliphatic amines, aromatic amines, amino acids, aminosugars, or peptides) is sonicated. Ter -polymeric pAA(Bacitracin;R) for which mol equiv of Bacitracin and RNH_2 to anhydride group of pAAn are >0 is generated in microtiter plates with 96 conically-bottomed wells as follows: (i) placing 3 mg of pAAn (M_n = 20700 gmol^{-1}, M_w = 39500 gmol^{-1}) into a well; (ii) soaking the powder with 10 µL of 0.1 M Bacitracin, and a variable amount (2-20 µL) of 0.1 M of RNH_2 (examples: naphthylalanine, phenylalanine, cyclohexylamine, phenylethylamine, 4-aminobenzoic acid, or mannosamine) in PBS buffer, pH 12; (iii) immediately sealing the plate, and then ultrasonicating the mixture for 0.5 h. Each solution of ter-polymers (pH ~ 3) generated in a well is neutralized to pH ~ 7 by adding 30 µL of 1.0 M NaOH and adjusted to 100 or 200 µL (total volume) with PBS, pH 7.2, before the inhibition assay of bacterial growth.

It is to be understood that the above description is intended to be illustrative and not restrictive. Many embodiments will be apparent to those of skill in the art upon reading the above description. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled. The disclosures of all articles and references, including patent applications and publications, are incorporated herein by reference for all purpose.

APPENDIX

Scheme I
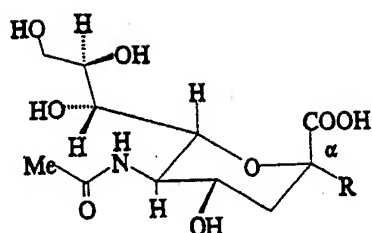
NeuAc R = OH; *N*-Acetylneuraminic Acid or Sialic Acid
  1   R = (CH$_2$)$_3$ S(CH$_2$)$_2$NH$_2$; NeuAc-L$_1$-NH$_2$
  2   R = O(CH$_2$)$_2$ O(CH$_2$)$_2$NH$_2$; NeuAc-L$_2$-NH$_2$

  3   R =

NeuAc-L$_3$-NH$_2$

  4   R =

NeuAc-L$_4$-NH$_2$

Table III. The hemagglutination inhibition activities of
pAA(NeuAc-L) and libraries of pAA(NeuAc-L; R).

Polymer	mol. eq. RNH$_2$		mol. eq. NeuAc-L-NH$_2$	K$_i^{HAI}$(μM)[a]
pAA(1)			0	15000[b]
			0.04(1)	27
			0.06(1)	13
			0.08(1)	3.9
			0.10(1)	3.4
			0.11(1)	4.4
			0.12(1)	1.1
			0.14(1)	1.1
			0.17(1)	0.50
			0.21(1)	0.30
pAA(2)			0.11(2)	0.80
pAA(3)			0.11(3)	0.20
pAA(4)			0.11(4)	3.1
pAA(1;R)	0.12(RNH$_2$)		0.10(1)	
	RNH$_2$			
	3-aminobenzoic acid			1.5
	3-amino-5-hydroxybenzoic acid			3.1
	4-aminobenzoic acid			3.1
	4-amino-2-hydroxybenzoic acid			3.1
	4-aminobenzenesulfonic acid			1.5
	2-aminonicotinic acid			1.5
	N-methylhydroxylamine			1.5
	(D)-2-amino-2-deoxyglucose			2.2
	(D)-2-amino-2-deoxymannose			0.055
	1-amino-1-cyclopropanecarboxylic acid			1.1
	1-amino-1-cyclopentanecarboxylic acid			0.20
	1-amino-1-cyclohexanecarboxylic acid			0.028
	aminocyclohexane			0.0043
	(L)-arginine			1.5
	(L)-glutamate			2.5
	(L)-histidine			1.5
	(D)-4-hydroxyproline			1.5
	(DL)-leucine			0.30
	(L)-phenylalanine			0.024
	(L)-4'-nitrophenylalanine			0.048
	(L)-phenylalanine methyl ester			0.024
	1-amino-2-phenylethane			0.0021
	(L)-3-(2'-naphthyl)alanine			0.00050
	(L)-tryptophan			0.0043
	(L)-Gly-(L)-Gly-(L)-Gly			3.1
	(L)-Gly-(L)-Phe			1.5
pAA(3;R)	0.13 (RNH$_2$)	0.11(3)		
	RNH$_2$			
	1-amino-2-phenylethane			0.0015
	(L)-3(2'-naphthyl)alanine			0.00070

Table IV. Inhibition of ricin-induced agglutination of chick erythrocytes by polyvalent polymeric galactosides

Inhibitor	K_i^{HAI} (μM)[a]		Inhibitor	K_i^{HAI} (μM)[a]	
	RCA$_{120}$	RCA$_{60}$		RCA$_{120}$	RCA$_{60}$
Gal-β-OMe	200	42	pBMA(Gal-β; 0)	>30000[b,c]	≥30000[b,c]
Gal-α-OMe	400	50	pBMA(Gal-β; 0.05)	2.0	1.0
Gal-β_o-L$_1$NH$_2$	37	16	pBMA(Gal-β; 0.09)	14	12
Gal-α_t-L$_2$NH$_2$	250	39	pBMA(Gal-β; 0.17)	5.0	>80
GlcNAc-β_o-L$_1$NH$_2$	>9000[b]	>9000[b]	pBMA(Gal-β; 0.22)	0.73	≥94
			pBMA(Gal-α; 0.05)	>160	≥160
PAA(Gal-β; 0)	>35000[b,c]	>35000[b,c]	pBMA(Gal-α; 0.09)	280	33
PAA(Gal-β; 0.2)	0.16	8.1	pBMA(Gal-α; 0.17)	61	17
PAA(Gal-β; 0.4)	0.14	17	pBMA(Gal-α; 0.22)	12	5.0
PAA(Gal-β; 0.6)	0.18	20	pAA(GlcNAc-β; 0.2)	>290[b]	>290[b]
PAA(Gal-β; 0.8)	0.27	42	pAA(NeuAc-α; 0.2)	>170[b]	>170[b]
PAA(Gal-β; 1.0)	0.56	180	pBMA(NeuAc-α; 0.2)	>200[b]	>200[b]

[a] The value of K_i^{HAI} refers to the lowest concentration of carbohydrate-containing side chains of the polymer that could inhibit ricin-induced agglutination of chick erythrocytes. Each value respresents an average of five independent measurements; the experimental uncertainty in each value is approximately ±50%.

[b] No inhibition was observed at the indicated concentrations.

[c] This value represents the concentration of carboxylic acid side chains of the polymer in solution that could inhibit ricin-induced agglutination of chick erythrocytes.
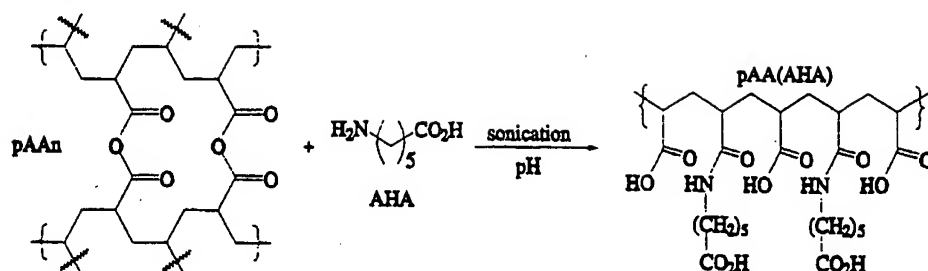
Equation 1.1a
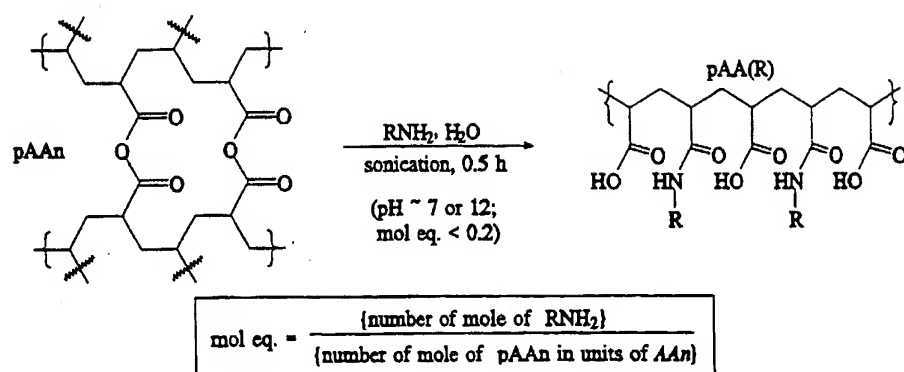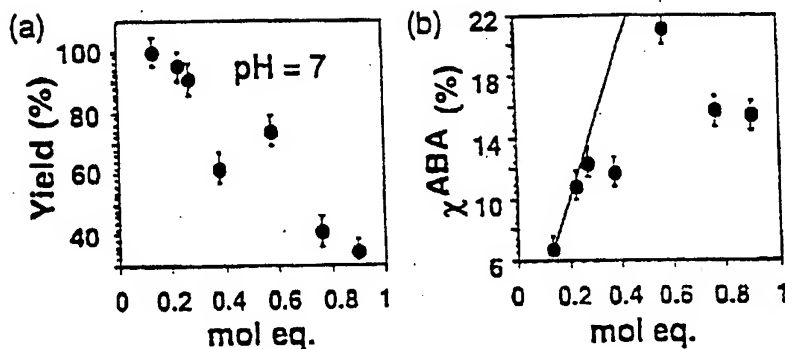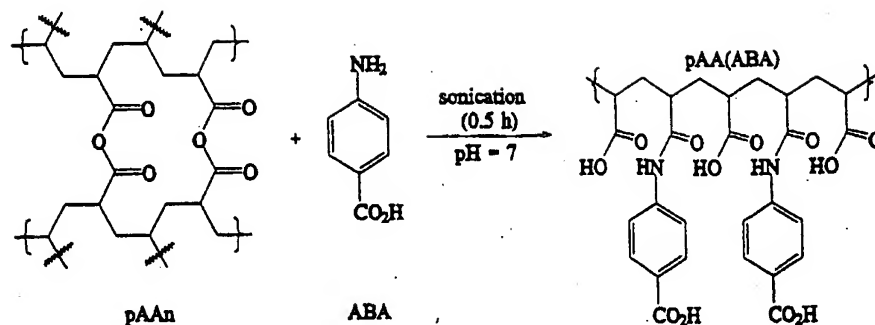
Equation 1.1b

(a)   A plot of yield of incorporation (%)# versus the number of molar

equivalents (mol eq.)* in reactions between 4-aminobenzoic acid (ABA)

and poly(acrylic anhydride) (pAAn) at pH 7. The covalent incorporation

of ABA into the side chains of poly(acrylic acid) (pAA) led to generation

of co-polymers, pAA(ABA).

(b)   A plot of mole fraction of ABA (χ^{ABA})† of pAA(ABA) versus the number

of molar equivalents: this plot was derived from plot 1.1(a). The straight

line in plot 1.1(b) is a reference line that represents quantitative reaction

(100% yield of incorporation).

$$\text{\#yield of incorporation} = \frac{\{\text{number of moles of } HO_2C-\langle \rangle-NHCO- \text{ from pAA(ABA)}\}}{\{\text{number of moles of } HO_2C-\langle \rangle-NH_2 \text{ used in a reaction}\}} \times 100(\%)$$

$$\text{*molar equivalent} = \frac{\{\text{number of moles of } HO_2C-\langle \rangle-NH_2 \text{ used in a reaction}\}}{\{\text{number of moles of used pAAn in units } f\langle \rangle\}}$$

$$\text{†mole fraction of ABA} = \chi^{ABA} (\%)$$

$$= \{\text{number of molar equivalents}\} \times \{\text{yield of incorporation \%}\}$$
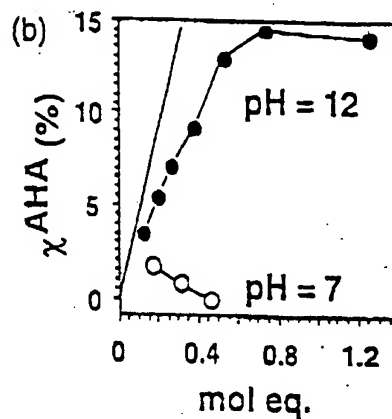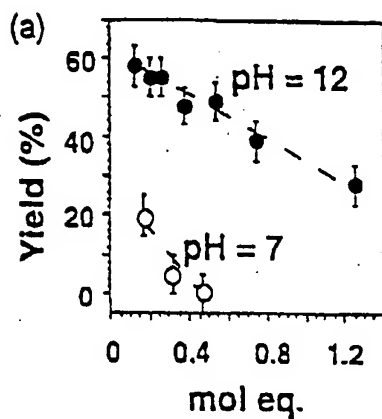
Equation 1.1c

(a)   A plot of yield of incorporation (%)$^#$ versus the number of molar equivalents (mol eq.)* in reactions between 6-aminohexanoic acid (AHA) and pAAn at pH 7 or 12.

(b)   A plot of mole fraction of AHA(χ^{AHA})† of pAA(AHA) versus the number of molar equivalents: this plot was derived from plot 1.2(a). The straight line in plot 1.2(b) is a reference line that represents quantitative reaction (100% yield of incorporation).

yield of incorporation = {number of moles of $HO_2C(CH_2)_5NHCO-$ from pAA(AHA)}
{number of moles of $HO_2C(CH_2)_5NH_2$ used in a reaction}

Equation 1.2

$$\text{pAAn} + \text{NeuAc-L}_1\text{-NH}_2 \xrightarrow[\text{pH}]{\text{sonication}}$$

pAA(NeuAc-L_1)

(a)

(b)
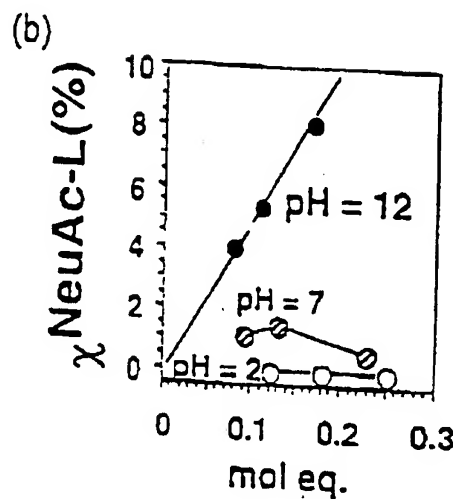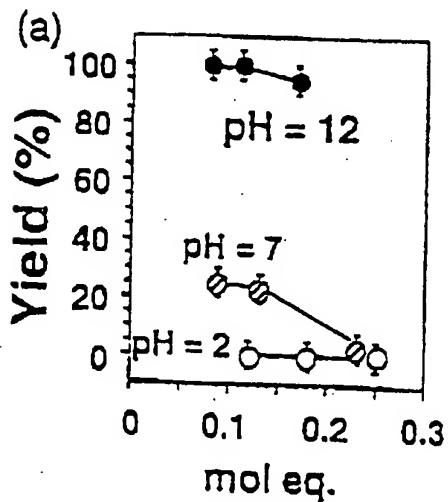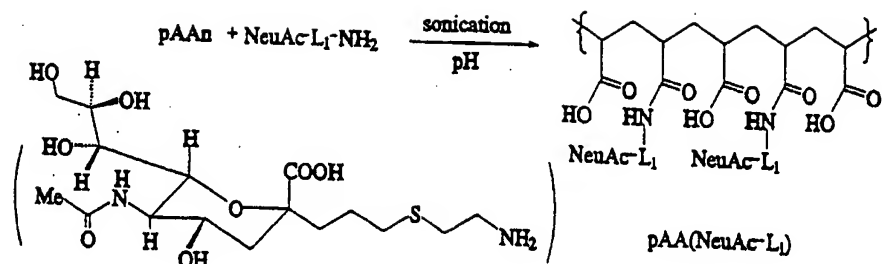
$$\text{\#yield of incorporation} = \frac{\{\text{number of moles of NeuAc-L}_1\text{-NHCO- from pAA(NeuAc-L}_1)\}}{\{\text{number of moles of NeuAc -L}_1\text{-NH}_2 \text{ used in a reaction}\}} \times 100(\%)$$

$$\text{*molar equivalent} = \frac{\{\text{number of moles of NeuAc-L}_1\text{-NH}_2 \text{ used in a reaction}\}}{\{\text{number of moles of used pAAn in units of} \qquad \}}$$

$$\dagger\text{mole fraction of NeuAc-L}_1 = \chi^{\text{NeuAc-L}_1} (\%)$$

$$= \{\text{number of molar equivalents}\} \times \{\text{yield of incorporation }\%\}$$

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1. A method of making an array of polyvalent presenters, said method comprising:

(a) delivering a first activated framework component of a first polyvalent presenter and a first activated framework component of a second polyvalent presenter to first and second reaction vessels; and

5

(b) delivering a first functional group component of said first polyvalent presenter and a first functional group component of said second polyvalent presenter to said first and second reaction vessels; thereby forming an array of at least two different polyvalent presenters;

10

said method optionally further comprising one ore more of the following steps:

(c) delivering a first spacer group of said first polyvalent presenter to said first reaction vessel;

(d) delivering a first spacer group of said second polyvalent presenter to said second reaction vessel;

15

(e) delivering an ancillary group of said first polyvalent presenter to said first reaction vessel;

(f) delivering a second functional group component of said first polyvalent presenter to said first reaction vessel;

20

(g) delivering a third functional group component of said first polyvalent presenter to said first reaction vessel;

(h) delivering a second functional group component of said second polyvalent presenter to said second reaction vessel;

(i) delivering a third functional group component of said second polyvalent presenter to said second reaction vessel; or

25

(j) screening said array of polyvalent presenters for a useful property (optionally wherein said useful property is selected from the group consisting of biological activity, binding affinity, biological properties, pharmacological properties, oral bioavailability, circulatory half-lives, agonist activity, antagonist activity and solubility);

30

said method comprising one ore more of the following optional variations:

(1) selecting said first activated framework component of said first polyvalent material and first activated framework component of said second polyvalent material to be the same, but offering them in different amounts;

35

(2) selecting said first activated framework component of said first polyvalent material and said first activated framework component of said second polyvalent material to be different;

(3) selecting said first functional group component of said first polyvalent presenter and said first functional group component of said second polyvalent presenter to be the same, but offering them in different amounts;

40

(4) selecting said first functional group component of said first polyvalent presenter and said first functional group component of said second polyvalent presenter to be different; or

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(5) selecting said framework components to comprise a liposome or a liposome derivative, a micelle, a colloid, a biological particle selected from the group consisting of a protein aggregate, a cell, a sugar, a protein, a lipid and a small molecule, a dendrimer, a polymer, optionally selected from the group consisting of homopolymers and copolymers.

2. The method as recited in claim 1 wherein said delivery of said first spacer group of said first polyvalent presenter occurs prior to, or simultaneously with, step (b).

3. The method as recited in claim 1 wherein said delivery of said ancillary group of said first polyvalent presenter occurs prior to, or simultaneously with, step (b).

4. The method as recited in claim 1 wherein said delivery of said second functional group component of said first polyvalent presenter occurs simultaneously with, or after, step (b).

5. The method as recited in claim 1 wherein the stoichiometry of said first activated framework component to said first functional group component of said first polyvalent presenter is 1:1.

6. The method as recited in claim 1 wherein said first functional group component of said first polyvalent presenter is delivered in an amount sufficient to consume all of the activated groups of said first activated framework component.

7. The method as recited in claim 1 wherein said first functional group component of said first polyvalent presenter is delivered in an amount insufficient to consume all of the activated groups of said first activated framework component.

8. The method as recited in claim 1 wherein said delivery of said second functional group component of said second polyvalent presenter occurs simultaneously with, or after, step (b).

9. The method as recited in claim 1 wherein a second functional group component of said first polyvalent presenter is delivered to said first reaction vessel, said second functional group component optionally being a mixture of functional groups components.

10. The method as recited in claim 7 wherein an ancillary group of said first polyvalent presenter is delivered to said first reaction vessel, said ancillary group optionally being a mixture of ancillary groups.

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11. The method as recited in claim 7 wherein a second functional group and an ancillary group of said first polyvalent presenter are delivered to said first reaction vessel.

5   12. The method as recited in claim 1 wherein after the attachment of said first functional group component to said first activated framework component of said first polyvalent presenter, said first activated framework component is reactivated and a second functional group component is delivered to said first reaction vessel, said second functional group component optionally being a mixture

10   of functional group components.

13. The method as recited in claim 1 wherein after the attachment of said first functional group component to said first activated framework component of said first polyvalent presenter, said first activated framework component is

15   reactivated and an ancillary group is delivered to said first reaction vessel, said ancillary group optionally being a mixture of ancillary groups.

14. The method as recited in claim 1 wherein after the attachment of said first functional group component to said first activated framework component of

20   said first polyvalent presenter, said first activated framework component is reactivated and a second functional component and an ancillary group are delivered to said first reaction vessel.

15. The method as recited in claim 1 wherein said first spacer group of

25   said first polyvalent presenter forms a first linkage with said first activated framework component and a second linkage with said first functional group component; and optionally said first linkage and said second linkage of said first spacer group of said first polyvalent presenter being different; or further optionally said first spacer group of said first polyvalent presenter comprising a hydrolyzable

30   linkage, a biodegradable linkage, a cleavable linkage, a hydrophilic radical, or a radical of which at least a portion is comprised of a member selected from the group consisting of polyethylene glycol and substituted polyethylene glycol.

16. The method as recited in claim 1 wherein said first spacer group of

35   said second polyvalent presenter forms a first linkage with said first activated framework component and a second linkage with said first functional group component; and optionally said first linkage and said second linkage of said first spacer group of said second polyvalent presenter being different.

40   17. The method as recited in claim 15 wherein said hydrolyzable linkage is between said first linkage and said second linkage of said first spacer group.

18. The method as recited in claim 15 wherein said biodegradable linkage is between said first linkage and said second linkage of said first spacer group.

5   19. The method as recited in claim 15 wherein said cleavable linkage is between said first linkage and said second linkage of said first spacer group.

20. The method as recited in claim 15 wherein said polyethylene glycol portion has a formula weight ranging from about 100 to about 20,000,
10   optionally from about 200 to about 1,000, Daltons.

21. The method as recited in claim 15 wherein said first spacer group is a member selected from the group consisting of

15          $X-R^6-Y-R^7-Z$, $X-R^7-Y-R^6-Z$ and $X-R^6-Z$;

in which:
     R^6 is polyethylene glycol having a formula weight of from about 100 to about 20,000 Daltons;
20   R^7 is a member selected from the group consisting of S-S, C(O)-O, and O-C(O); and
     X, Y, and Z are the same or different and are inert linking groups.

22. The method as recited in claim 21 wherein X, Y, and Z are the
25   same or different and are each a member selected from the group consisting of $(CH_2)_q-NH$, $NH-(CH_2)_q$, $NH-C(O)-O$, $O-C(O)-NH$, $(CH_2)_q-NH-C(O)-O$ and $O-C(O)-NH-(CH_2)_q$, in which q is 1 to 10.

23. The method as recited in claim 22 wherein q is 2 to 4, optionally 2.
30   24. The method as recited in claim 15 wherein said first spacer group has the general formula

          $X-R^6-Y'(-R^7-Z)_r$
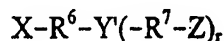
35   in which:
     R^6 is polyethylene glycol having a formula weight of from about 100 to about 20,000 Daltons;
     R^7 is a member selected from the group consisting of S-S, C(O)-O, and O-C(O);
40

X is NH-C(O)-O;

Y' is O-C(O)-NH-(CH$_2$)$_q$-CH$_3$ in which q is 1 to 3, and a number equal to m of the H atoms bonded to the C atoms are substituted by NH-(CH$_2$)$_s$-NH where s is 2 to 4;

Z is (CH$_2$)$_q$-NH in which q is 2 to 4; and

r is zero or m.

25. The method as recited in claim 24 wherein r is m; and optionally m is 2 to 6, optionally m is 2.

26. The method as recited in claim 1 wherein said polymer comprises a poly(ester) or a derivative thereof, a poly(anhydride) or a derivative thereof, a carbohydrate or a derivative thereof, a polyol or a derivative thereof, a poly(acrylate) or a derivative thereof, a poly(methacrylate) or a derivative thereof, a poly(ether) or a derivative thereof, a poly(amino acid) or a derivative thereof.

27. The method as recited in claim 1 wherein said polymer comprises a derivative of a compound selected from the group consisting of poly(glutamic acid), poly(aspartic acid), dextran, dextran sulfate, poly(maleic anhydride-co-vinyl ether), poly(succinimide), poly(acrylic anhydride), poly(ethylene glycol), poly(lactic acid), poly(glycolic acid), poly(vinyl pyrrolidine), poly(styrene-maleic anhydride), poly (alpha-olefin-maleic acid), hyaluronic acid, sodium carboxymethylcellulose, chondroitin sulfate, poly(methacrylate), poly(acrylate), poly(acrylamide), poly(glycerol) and starch.

28. The method as recited in claim 1 wherein said first functional group component promotes targeting of said first polyvalent presenter.

29. The method as recited in claim 28 wherein said first functional group component of said first polyvalent presenter is used to facilitate visualization of the target cell.

30. The method as recited in claim 1 wherein said first functional group component of said first polyvalent presenter comprises a therapeutic agent..

31. The method as recited in claim 1 wherein said first functional group component of said first polyvalent presenter is macromolecular.

32. The method as recited in claim 1 wherein at least 10, at least 50, or at least 100, or at least 1,000, or at least 10,000, or at least 100,000, or at least 1,000,000 different polyvalent presenters are made.

33. The method as recited in claim 1 wherein each of said polyvalent presenters is water soluble, optionally in the milligram/milliliter range, or gram/milliliter range.

5   34. A method of preparing a polyvalent presenter or an array of polyvalent presenters having a useful property, said method comprising the steps of:

(i) making an array of polyvalent presenters as recited in any one of claims 1 to 33; and

(ii) screening said array of polyvalent presenters for a useful property to

10   identify a polyvalent presenter having said useful property; and optionally

(iii) separating a polyvalent presenter having said useful property from said array of polyvalent presenters.

35. A method of making an array of polyvalent presenters, said

15   method comprising:

(a) delivering a first monomer of a first polyvalent presenter and a first monomer of a second polyvalent presenter to first and second reaction vessels;

(b) delivering a first functional monomer of said first polyvalent presenter and a first functional monomer of said second polyvalent presenter to said first and

20   second reaction vessels; and

(c) copolymerizing said first monomer and said first functional monomer of said first polyvalent presenter and said first monomer and said first functional monomer of said second polyvalent presenter, thereby forming an array of at least two different polyvalent presenters; wherein optionally said first monomer of said

25   first polyvalent presenter is derivatized with an ancillary group or a first spacer group.

36. The method as recited in claim 35 wherein said first monomer of said first polyvalent presenter is derivatized with a first spacer group that is

30   derivatized with an ancillary group.

37. The method as recited in claim 35 wherein the functional group of said first functional monomer of said first polyvalent presenter is attached through a first spacer group.

35

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38. The method as recited in claim 35 wherein said monomer is selected from the group consisting of acrylic-based monomers, optionally selected from the group consisting of acrylamide, acrylic acid, acrylonitrile and methyl acrylate; vinyl-based monomers, optionally selected from the group consisting of
5   vinylidene chloride, vinyl acetate, vinyl chloride, 2-vinylpyridine, vinyl laurate, vinylpyrrolidone, vinyl ethyl ether, vinyl fluoride; styrene, p-chlorostyrene, butadiene, maleic anhydride, epichlorohydrin, amino acids, nucleic acids, saccharides, diaminoalkanes, optionally selected from the group consisting of 1,4-diaminobutane and 1,2-diaminoethane.
10

39. The method as recited in claim 35 wherein said functional monomer is selected from the group consisting of acrylic-based functional monomers, optionally selected from the group consisting of acrylamide functional monomers, acrylic acid functional monomers, acrylonitrile functional monomers and
15   methyl acrylate functional monomers; vinyl-based functional monomers, optionally selected from the group consisting of vinylidene chloride functional monomers, vinyl acetate functional monomers, vinyl chloride functional monomers, 2-vinylpyridine functional monomers, vinyl laurate functional monomers, vinylpyrrolidone functional monomers, vinyl ethyl ether functional monomers and vinyl fluoride
20   functional monomers; styrene functional monomers, p-chlorostyrene functional monomers, butadiene functional monomers, maleic anhydride functional monomers, epichlorohydrin functional monomers, amino acid functional monomers, nucleic acid functional monomers, saccharide functional monomers, diaminoalkane functional monomers, optionally selected from the group consisting of 1,4-diaminobutane
25   functional monomers and 1,2-diaminoethane functional monomers.

40. A method of preparing a polyvalent presenter or an array of polyvalent presenters having a useful property, said method comprising the steps of:
      (i) making an array of polyvalent presenters as recited in any one of claims 35
30   to 39; and
      (ii) screening said array of polyvalent presenters for a useful property to identify a polyvalent presenter having said useful property; and optionally
      (iii) separating a polyvalent presenter having said useful property from said array of polyvalent presenters.

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41. A method of making an array of polyvalent presenters, said method comprising:

(a) delivering a first monomer of a first polyvalent presenter and a first

5   monomer of a second polyvalent presenter to first and second reaction vessels;

(b) delivering a first functional monomer of said first polyvalent presenter and a first functional monomer of said second polyvalent presenter to said first and second reaction vessels; and

(c) copolymerizing said first monomer and said first functional monomer of

10 said first polyvalent presenter and said first monomer and said first functional monomer of said second polyvalent presenter; and

(d) delivering a second monomer or a second functional monomer of said first polyvalent presenter and a second monomer or a second functional monomer of said second polyvalent presenter to said first and second reaction vessels;

15 thereby forming an array of at least two different polyvalent presenters.

42. The method as recited in claim 41 wherein said second monomer of said first polyvalent presenter is derivatized with an ancillary group or a spacer group.

20

43. The method as recited in claim 41 wherein the functional group of said second functional monomer of said first polyvalent presenter is attached through a spacer group.

25   44. The method as recited in claim 41 wherein said monomer is selected from the group consisting of acrylic-based monomers, optionally selected from the group consisting of acrylamide, acrylic acid, acrylonitrile and methyl acrylate; vinyl-based monomers, optionally selected from the group consisting of vinylidene chloride, vinyl acetate, vinyl chloride, 2-vinylpyridine, vinyl laurate,

30 vinylpyrrolidone, vinyl ethyl ether, vinyl fluoride; styrene, p-chlorostyrene, butadiene, maleic anhydride, epichlorohydrin, amino acids, nucleic acids, saccharides, diaminoalkanes, optionally selected from the group consisting of 1,4-diaminobutane and 1,2-diaminoethane.

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45. The method as recited in claim 41 wherein said functional monomer is selected from the group consisting of acrylic-based functional monomers, optionally selected from the group consisting of acrylamide functional monomers, acrylic acid functional monomers, acrylonitrile functional monomers and methyl acrylate functional monomers; vinyl-based functional monomers, optionally selected from the group consisting of vinylidene chloride functional monomers, vinyl acetate functional monomers, vinyl chloride functional monomers, 2-vinylpyridine functional monomers, vinyl laurate functional monomers, vinylpyrrolidone functional monomers, vinyl ethyl ether functional monomers and vinyl fluoride functional monomers, styrene functional monomers, p-chlorostyrene functional monomers, butadiene functional monomers, maleic anhydride functional monomers, epichlorohydrin functional monomers, amino acid functional monomers, nucleic acid functional monomers, saccharide functional monomers, diaminoalkane functional monomers, optionally selected from the group consisting of 1,4-diaminobutane functional monomers and 1,2-diaminoethane functional monomers.

46. A method of preparing a polyvalent presenter or an array of polyvalent presenters having a useful property, said method comprising the steps of:
        (i) making an array of polyvalent presenters as recited in any one of claims 41 to 45; and
        (ii) screening said array of polyvalent presenters for a useful property to identify a polyvalent presenter having said useful property; and optionally
        (iii) separating a polyvalent presenter having said useful property from said array of polyvalent presenters.

47. An array of more than two, more than 10, more than 50, more than 100, more than 1,000, more than 10,000, more than 100,000, or more than 1,000,000 different polyvalent presenters.

48. The array as recited in claim 47 wherein each said polyvalent presenters is on a substrate at known locations.

49. The array as recited in claim 48 wherein said substrate is a single substrate.

50. The array as recited in claim 48 wherein said substrate is a 96-well microtiter plate.

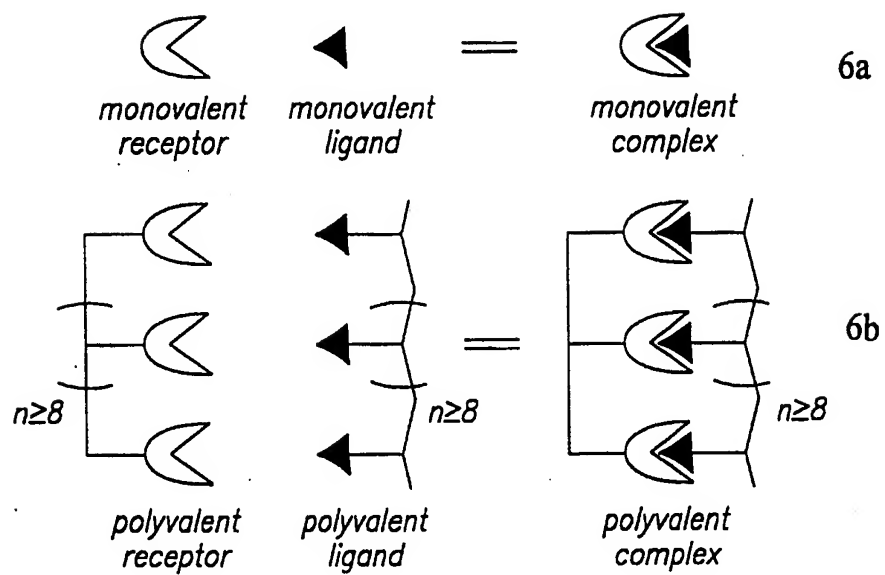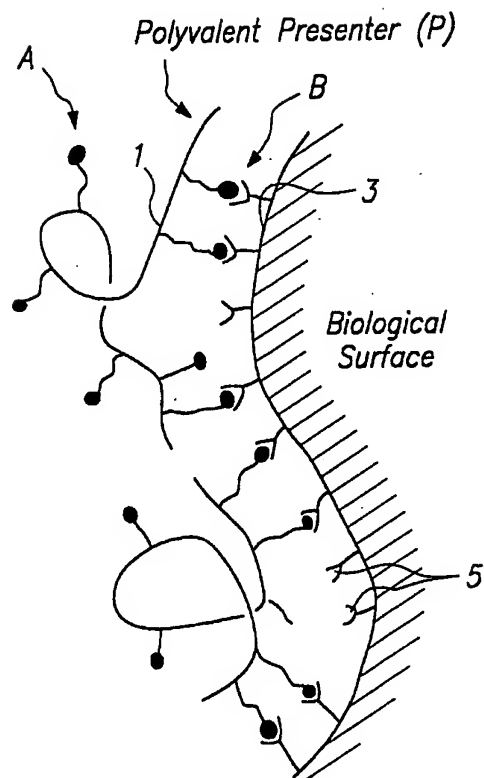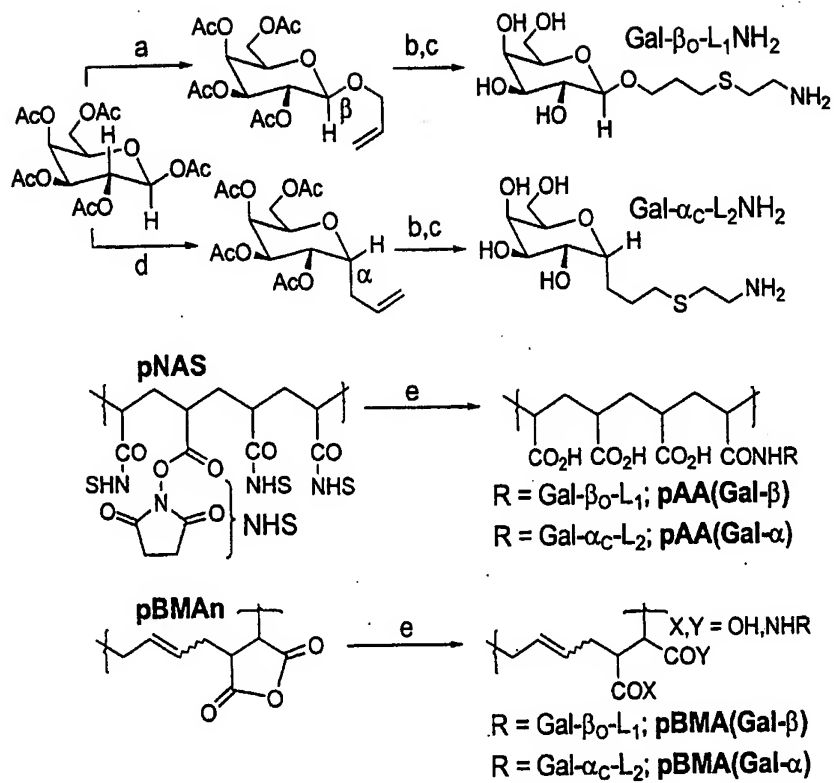51. An array of polyvalent presenters preparable by any one of the methods recited in claims 1 to 46.

FIGURE 1

monovalent
receptor

monovalent
ligand

=

monovalent
complex

6a

$n \geq 8$

polyvalent
receptor

$n \geq 8$

polyvalent
ligand

=

$n \geq 8$

polyvalent
complex

6b

FIGURE 2

3/6

FIGURE 3

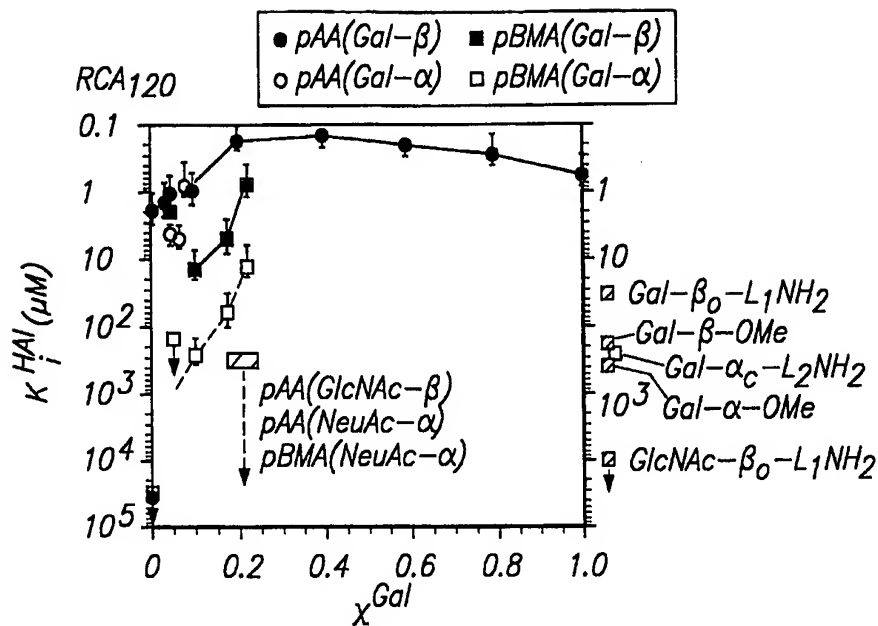$$\text{mole fraction of R of pAA(R) or pBMA(R)} = \chi^R$$

$$\chi^R = \frac{[-CONHR]}{[-CO_2H] + [-CONHR]}$$

FIGURE 4a

FIGURE 4b

FIGURE 5

χ = mole fraction of R of pAA(R;χ) or pBMA(R;χ)

$$\chi^R = \frac{[-CONHR]}{[-CO_2H] + [-CONHR]}$$

FIGURE 6

Sialyl Lewis x mimetic
(SLe^x-LNH$_2$)

6a SLe^x-NH$_2$, H$_2$O, pH 12
sonication, 30 min

pAAn

R = SLe^x-LNH$_2$; **pAA(SLe^x;χ)**

6b pAAn Bacitracin, H$_2$O, pH 12
sonication, 30 min

CO$_2$H CO$_2$H CO$_2$H CONHR

R = Bacitracin; **pAA(bacitracin;χ)**

χ = mole fraction of R of pAA(R;χ), pMVMA(R;x), or pBMA(R;χ)

$$\chi^R = \frac{[\text{-CONHR}]}{[\text{-CO}_2\text{H}] + [\text{-CONHR}]}$$